Division of labour and the evolution of complex social groups

Guy Alexander Cooper

A thesis submitted for the degree of *Doctor of Philosophy*

Trinity term 2018

Epigraph

"I…will confine myself to one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory. I allude to the neuters or sterile females in insect communities: for these neuters often differ widely in instinct and in structure from both the male and fertile females, and yet from being sterile they cannot propagate their kind."- *The Origin of Species*, Charles Darwin

"It is the great multiplication of the productions of all the different arts, in consequence of the division of labour, which occasions, in a well-governed society, that universal opulence which extends itself to the lowest ranks of the people."- *The Wealth of Nations*, Adam Smith

"And how will they proceed? Will each bring the result of his labours into a common stock?—the individual husbandman, for example, producing for four, and labouring four times as long and as much as he need in the provision of food with which he supplies others as well as himself; or will he have nothing to do with others and not be at the trouble of producing for them, but provide for himself alone a fourth of the food in a fourth of the time, and in the remaining three fourths of his time be employed in making a house or a coat or a pair of shoes, having no partnership with others, but supplying himself all his own wants?"- *The Republic*, Plato.

Declaration

I declare that this work was composed by myself and that the work contained herein is my own except where explicitly stated in the text. This work has not been submitted for any degree or professional qualification except as specified.

Guy Alexander Cooper, Trinity term 2018

Acknowledgments

Thank you to my collaborators and to any and all who gave me feedback on various iterations of papers, practice talks, posters, and chapter drafts: Ashleigh Griffin, Alan Grafen, Jay Biernaskie, Tobi Kiers, Charlie Cornwallis, Koos Boomsma, Geoff Wild, Daniel Unterweger, Philip Downing, Sam Levin, John Bruce, Berti Fisher, Tom Scott, Asher Leeks, and Miguel dos Santos. Thanks to Andy Gardner and Kevin Foster for being great thesis examiners. I would like to acknowledge the support and friendship of the various affiliates of E7, both pre- and post-Tinbergen exodus: Philip, Shana, Berti, Sam, Tom, Miguel, Asher, Gijsbert, Ornela, Mati, and Max. Thank you to the community of Holywell Manor: Calum, Mitch, Deeps, Robin, Vassili, Sarah, Ioanna, Nina, Chris, Raag, Giulio, Megan, Jess, Fabio, Emily, Will, Helen, Pierre, and Rob. You made Oxford my home and I consider you all my family. To Mitch, Jess, and Megan, the residents of Abbey Road, thank you for your kindness, support and friendship.

I am grateful to Dave Gavaghan and the DTC for giving me this opportunity. In particular, I would like to thank the EPSRC for the funding that made it all possible. I am also grateful for the support of Balliol College.

To my supervisor, Stu: words cannot express how lucky I feel to have had your generous guidance and unqualified support over the last few years. Thank you for not making fun of me for citing Plato.

I would like to thank my brother, Ian, for his support in all matters technology and media. To Mom and Dad: thank you for providing me with so many amazing opportunities in life- I am eternally grateful. Phoebe, thank you for all of the things.

Contribution and publications

I have included my contribution to the sections of each chapter in brackets, given as a percent contribution for each element.

The following published paper arose from this thesis and is presented in Chapter 2. The paper in its published format is included in the supplementary materials at the end of the thesis. Stuart West and I conceived of the idea for the paper (50%). I developed the mathematical model and performed the analysis (100%). Stuart West and I wrote the paper together (50%).

- Cooper, G. A., and West, S. A. (2018) Division of labour and the evolution of extreme specialisation. *Nature Ecology and Evolution*. 2, 1161–1167

Chapters 3 and 4 are my own work supervised by Stuart West. Stuart West and I conceived of each project together (50%). I developed the mathematical models and performed the analyses (100%) and I wrote the manuscripts with Stuart West (50%). Each chapter is currently being prepared to submit for publication.

Chapter 5 is a published opinion piece that I wrote with Sam Levin, Geoff Wild and Stuart West. The article in its published format is included in the supplementary materials at the end of the thesis. Stuart West and I conceived of the piece (50%) and Sam Levin and I were the principle writers of the manuscript (50%).

- Cooper, G. A., Levin, S. R, Wild, G. and West, S. A. (2018) Modelling relatedness and demography in social evolution. *Evolution Letters*. 2(4), 260–271

The supplementary materials also include an unpublished manuscript, 'defensive group formation and the evolution of multicellularity,' which is not included as a chapter of the thesis for thematic coherence. Stuart West and I conceived of the project (50%) and I developed and analyzed the mathematical models (100%). Stuart West and I wrote the manuscript together (50%).

I also contributed to two other published papers, which are included in the supplementary materials.

The first is a perspective written with Stu West. I was not involved in conceiving of the study but I was involved in writing the manuscript (50%) and I designed the figures (100%).

- West, S. A., and Cooper, G. A. (2018) Division of labour in microorganisms: an evolutionary perspective. *Nature Reviews Microbiology*. 14, 716–723

The second was a collaborative work with John Bruce *et* al.. I was not involved in conceiving the study, nor did I contribute to the collection and analysis of the data. However, Stuart West and I conceived of the mathematical model (50%), I performed the mathematical analysis (100%), and I contributed a written section to the final manuscript (25%).

- Bruce, J. B., Cooper, G. A., Chabas, H., West, S. A. and Griffin, A. S. (2017) Cheating and resistance to cheating in natural populations of the bacterium Pseudomonas fluorescens. *Evolution*. *71*(10), 2484-2495.

Abstract

Division of labour occurs when cooperating individuals specialise to perform different roles. This kind of behaviour is encountered widely across the tree of life, from the separate functions of distinct genes within a genome to the specialised castes of eusocial insect societies. However, division of labour is not simply a ubiquitous social behaviour across the natural world. Instead, it has also been a critical factor in the evolution of more and more complex forms of life.

When individuals divide labour, they become more dependent upon the cooperation of others. When the mutual dependence scales tip such that individuals can no longer survive and reproduce outside the group, then a transition in individuality may occur such that the group itself begins to act as an evolutionary individual in its own right. For example, the division of labour between reproductive germ cells and sterile somatic cells facilitated the evolution of obligate multicellular organisms. A similar division between reproductive queens and sterile workers underlies the evolution of eusocial insect societies. As a consequence, the study of division of labour not only provides explanations for a diverse behaviour but can also help us to understand why some forms of life have become so complex.

In this thesis, I have employed kin-selection theory to explain the evolution of division of labour between helpers and reproductives. I first show when such division of labour is favoured over uniform behaviour and draw conclusions about the degree of specialisation that is expected to evolve. I next consider the biological factors that can lead to the evolution of social groups comprised predominantly of sterile helpers.

I then develop evolutionary theory to explain why labour dividers may employ environmental information to optimally coordinate their allocation of labour. In much of this work, I consider the effect of relatedness on the evolution of division and its various forms. I finish the thesis with an opinion piece on the different ways that relatedness may be modelled in social evolution theory.

Contents

Chapter 1: Introduction

Defining division of labour in nature

The concept of division of labour was originally developed to describe behaviour within human societies. Its first known discussion is found in Plato's *Republic*, in which the Greek philosopher contemplated the duties that each person would perform in a state of the minimum possible size[*]. Nowadays, it is perhaps most frequently associated with Adam Smith's *The Wealth of Nations*, in which he argued that the most efficient allocation of labour within a pin-making factory would involve specialised workers that each excelled in a particular task in the production line. Smith also argued that different nations that invest in different industries and trade the surplus of their products may be said to have a division of labour (Smith, 1776). In all cases, division of labour involves different entities (individuals or nations) performing distinct tasks that make the wider group more efficient (the factory or international community).

Across the tree of life and at all levels of biological organisation, we encounter behaviours that seem similar to the labour dividing feats of humans (Bourke, 2011; Davies, Krebs and West, 2012). Within the bustle of an ant nest, castes of specialised workers perform distinct tasks such as foraging, nest-defence, or nursing of the queen's young (Oster and Wilson, 1978; Bourke, 2011). There is a separation of skills between sessile flowers, which specialise on nectar production, and winged bees, who spread pollen from plant to plant, enabling the flowers to reproduce (Bourke, 2011; Seeley, 2014). Within the bodies of animals, trillions of cells differentiate into specialised types, such as blood cells, muscle cells and nerve cells (Bourke, 2011; Tortora and Derrickson, 2014). Further down, there is

[*] For the record, a shoemaker makes the cut in a society of only 4 people.

a separation of tasks between the different organelles of each cell and, indeed, each of the cell's genes codes for different proteins that carry out distinct functions (Alberts *et al.*, 2008; Bourke, 2011).

Given that such behaviour is encountered across the breadth of the natural world, we ask what specifically constitutes biological division of labour. We propose here a definition of division of labour that requires three key conditions (West and Cooper, 2016):

1. different individuals within the division perform distinct actions or behaviours (phenotypic variation);
2. some of the distinct actions or behaviours of the specialised individuals are cooperative, and selected for because they positively impact the fitness of social partners (cooperation);
3. The allocation of labour between individuals (degree of specialisation or abundance of each type) is determined by the inclusive fitness benefits that each derive from the division (adaptation)[†].

Stipulating the presence of cooperative behaviours (condition 2) ensures that instances in which phenotypic variation has evolved simply due to the direct fitness imperatives of each phenotypic class are not classified as division of labour. For example, one would not say that different microbial groups within a population that each specialise on growth in different niches of the environment are dividing labour (West and Cooper, 2016). There

[†] We note that an individual cannot gain inclusive fitness benefits from the increased specialisation of another phenotypic class. However, this will affect the inclusive fitness of individuals of the same class. Further, the relative abundance of the different phenotypic classes may affect the inclusive fitness of all individuals, regardless of phenotype.

must exist some beneficial interactions between the involved parties. In accordance with this definition, a division of labour may either be non-reproductive or reproductive, depending on whether all or only some individuals engage in distinct cooperative behaviours, respectively (**Figure 1**).

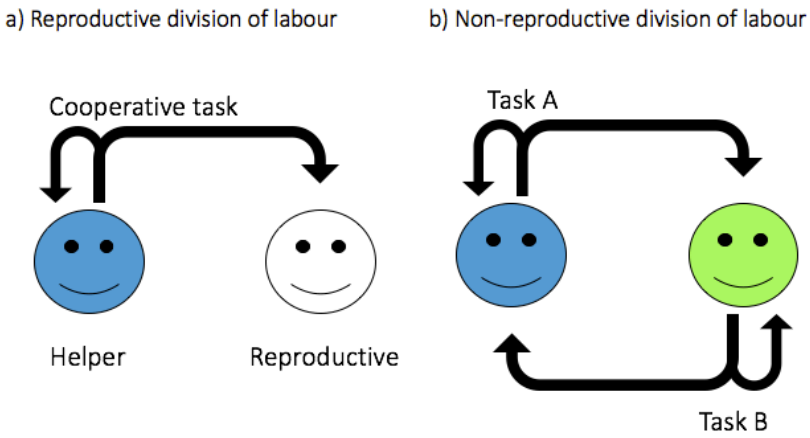


Figure 1: The two types of division of labour. a) In reproductive division of labour, some phenotypes engage in cooperative behaviours (helpers) whereas other do not engage in cooperation but marshal their resources for personal reproduction (reproductives). This constitutes division of labour so long as the helpers receive indirect fitness benefits through the help given to reproductives (condition 3). b) In non-reproductive division of labour, different phenotypes engage in different cooperative tasks and all individuals reproduce.

Condition 3 ensures that conflict is not the main driver of the joint phenotype (Queller and Strassmann, 2018). This precludes parasitism, cheating, or exploitation as examples of division of labour, because decreasing the proportion of the less cooperative individuals increases the relative inclusive fitness of the more cooperative individuals in these cases. For example, when a common cuckoo lays an egg in a reed warbler's nest, this does not qualify as division of labour (Davies and Brooke, 1988). Similarly, in human society, one would not consider the interaction of a pick-pocketer and an otherwise philanthropic individual as a division of labour. In these instances, the victim (helper) does not gain any fitness benefits (direct or indirect) by providing aid to the selfish individuals.

Our definition of division of labour emphasises the common purpose or goal that underlies individual specialisation (adaptation; conditions 2 and 3). This is an important stipulation for two reasons. First, it means that the use of intentional language is justified. Even though individuals may have no cognitive intent, the blind play of natural selection gives the appearance that each is consciously dividing up tasks with social partners and thus the comparison with the goal-oriented division of labour in humans is valid (Dawkins, 1976; Grafen, 1999; West, Griffin and Gardner, 2007; West and Cooper, 2016). Second, this definition groups together biological cases that both appear similar and that have evolved for the same reasons while precluding examples that may appear similar but have evolved for very different reasons (Maynard Smith, 1976). Thus, we may compare instances of specialisation in different species and fruitfully discuss the direct and indirect fitness benefits afforded by the adoption of complementary roles in each case. This facilitates the development of general evolutionary theory that can explain the many cases of division of labour with a common set of evolutionary principles.

Why should we care about division of labour?

A general body of theory that could explain why division of labour evolved in some cases but not others would do more than just provide an explanation for one kind of diversity in nature. This is because division of labour is not simply an incidental flourish of each level of biological organisation at which it is found. Instead, division of labour is an instrumental factor in driving the emergence of increasingly more complex biological forms (Bourke, 2011; West *et al.*, 2015; Cooper and West, 2018). This process is described in the study of major evolutionary transitions.

Complex life on Earth has evolved through a series of major evolutionary transitions in individuality (Szathmáry and Maynard Smith, 1995; Queller, 1997; Bourke, 2011; West *et al.*, 2015). In each case, previously independent evolutionary individuals (**Figure 2a**) came together to form a new kind of individual (**Figure 2b**), which then can only survive and reproduce as a whole (**Figure 2c**). Examples include when individual replicators (proto-genes) combined to form the first cells' genomes, when independent simple cells fused to form more complex cells, when free floating cells began to attach to one another to form large multicellular organisms, and when previously autonomous insects formed eusocial insect societies (Bourke, 2011).

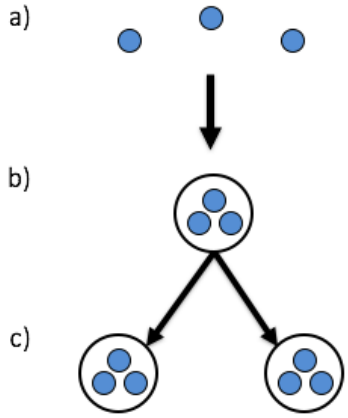


Figure 2: Major evolutionary transitions in individuality. A transition in individuality occurs when previously independent individuals (a) come together to form a group (b) that then can only reproduce or survive as a whole (c).

There are two different kinds of evolutionary transition in individuality, highlighting the different ways in which division of labour may be involved (Queller, 1997) (**Figure 3**). In fraternal transitions (**Figure 3a**), the constituent individuals are of the same species and there is a division of labour with some individuals becoming pure reproductives and others becoming sterile helpers. For instance, in the formation of multicellular animals, some cells became purely reproductive germ cells (sperm and egg) whereas as the rest became

sterile somatic cells (muscle cells, nerve cells, blood cells, etc.). Similarly, the formation of eusocial insect societies involved queens that specialise on brood production and sterile workers who instead perform specialised tasks for the hive. In contrast, egalitarian transitions (**Figure 3b**) occur when the constituent individuals belong to different species and all individuals retain reproductive capacities. Instead, egalitarian transitions involve a non-reproductive division of labour, in which each partner provides some benefit to the other. For instance, in the conjectured formation of the eukaryotic cell, the endosymbiotic prokaryote (mitochondria) may have been able to specialise on ATP production and the archaeal cell that surrounds it might have specialised on all other metabolic activities (Alberts *et al.*, 2008). Critically, the replicated genes of both cells are inherited upon cell division (no reproductive division of labour).

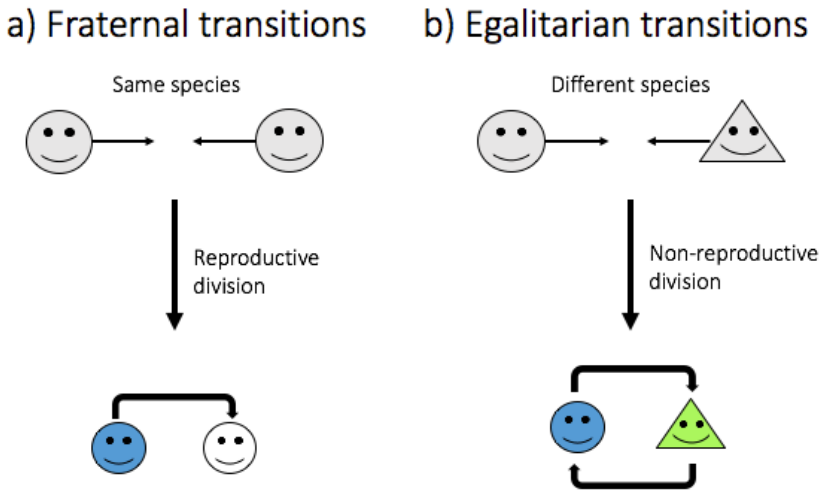


Figure 3: The different kinds of major transitions in indiviuality. a) Fraternal transitions occur when individuals of the same species form an obligate group and divide labour between sterile helpers and pure reproductives. Examples include the evolution of multicellular organisms and obligately eusocial insect socities. b) Egalitarian transitions occur when individuals of different species from an obligate group, specialize on distinct tasks, but where all individuals maintain reproductive capabilities. One example is the formation of the eukaryotic cell from a bacterium and an archaeon.

All transitions appear to have two key stages (Bourke, 2011; West *et al.*, 2015) (**Figure 4**). Group formation is the first stage and occurs when previously independent individuals

(**Figure 4a**) join together to form facultative group associations for cooperative benefits (**Figure 4b**). This can be facilitated by an environmental change. For example, *Dictyostelium discoideum* cells form a fruiting body when environmental nutrients are scarce (Strassmann, Zhu and Queller, 2000). Unicellular algae may come together to form defensive clumps as a response to predatory pressures (Boraas, Seale and Boxhorn, 1998). In both cases, individuals come together in some way in order to cooperate and share benefits.

The second key stage, group transformation, is facilitated by division of labour and occurs when the facultative group (**Figure 4b**) becomes an obligate and irreversible association (**Figure 4c**) (Bourke, 2011; West *et al.*, 2015). Division of labour is predicted to play a key role in driving this process because, when individuals specialise (or specialise further), they become increasingly dependent upon the complementary cooperation of other individuals within the group (Bourke, 2011; West *et al.*, 2015). In fraternal transitions, this involves the gradual separation into helper and reproductive roles (Queller, 1997, 2000). In egalitarian transitions, each individual begins to specialise further on the traits that it possesses, at the cost of proficiency in traits possessed by its partner (Queller, 1997). A tipping point occurs when extreme specialisation means that individuals cannot survive at all outside of the association, leading to full mutual dependence between group-mates and thus triggering obligate group formation. When this has occurred in groups with minimal conflict, the major transition is complete and a new kind of evolutionary individual has evolved (Gardner and Grafen, 2009; Bourke, 2011)(**Figure 4c**).

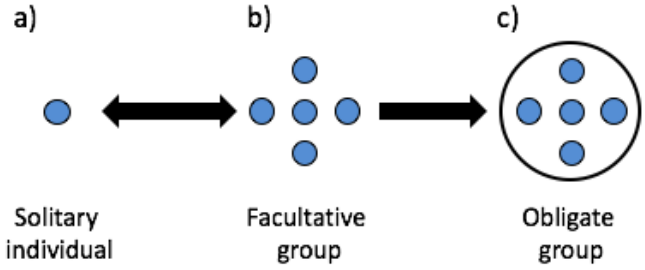


Figure 4: Group formation and group transformation. There are two key stages in an evolutionary transition in individuality. In the group formation stage, solitary individuals (a) from a facultative group (b) in response to an environmental change. This group structure may dissolve and return to solitary individuals (a) when the environment changes again. In the group transformation stage, the facultative group (a) evolves such that individuals can no longer survive and reproduce outside of the group, therefore leading to obligate group formation (c). Division of labour is predicted to play a role in the group transformation stage.

Consequently, if we want to understand why some lineages on the tree of life have undergone major evolutionary transitions whereas others have not, then we need to develop a general understanding of why individuals are sometimes selected to specialise and divide labour.

The connection to reproductive skew theory

A related field is reproductive skew theory, which attempts to explain why animal and insect societies vary in the degree to which some individuals may monopolize reproduction compared to others in the group (Vehrencamp, 1979; Johnstone, 2000; Reeve and Keller, 2001; Buston *et al.*, 2009). Societies with high skew are those in which only a few dominant individuals reproduce. Societies with low skew are those in which breeding is more uniformly distributed between all individuals. Consequently, this body of work may be connected to the study of reproductive division of labour as laid out above.

The litany of reproductive skew models may be broadly divided into three categories: concession models, restraint models, and compromise models, each involving different assumptions and leading to different predictions about the factors that may lead to high skew (Johnstone, 2000; Reeve and Keller, 2001). Concession and restraint models are both transactional in nature, relying on a trade-off between group stability and the relative inclusive fitness benefits captured by dominants and subordinates (Johnstone and Cant, 2010). In concession models, a dominant individual is in control of the allocation of reproduction within the group but the subordinates may leave to adopt a solitary lifestyle if they are not accorded sufficient inclusive fitness benefits (both direct and indirect) from group living (Vehrencamp, 1979; Johnstone and Cant, 2010). In restraint models, subordinates are in control of the allocation of reproduction within the group, but dominants may evict them if the dominants do not benefit sufficiently from group-living (Johnstone and Cant, 2010). In compromise models, neither party has full control over reproduction but they may differ in their intrinsic fighting or competitive ability and the final allocation of reproduction arises as the outcome of their antagonistic competition to reproduce (Johnstone and Cant, 2010). A more recent set of models, synthetic models, has attempted to construct meta-theory in which the previous three categories of model arise as different areas of parameters space (Buston *et al.*, 2009; Johnstone and Cant, 2010).

The common assumptions of all of these models reveal that the proposed mechanism for the emergence of reproductive skew may in fact not qualify as division of labour. These models satisfy the first two conditions for division of labour: there is a separation into distinct roles (dominant and subordinates; condition 1) and all models include a cooperative benefit to group formation (condition 2). However, in all models, the stable level of reproductive skew arises from the resolution of a conflict between opposing

parties (Vehrencamp, 1979; Queller and Strassmann, 2018). At a fixed level of group productivity, all individuals are attempting to increase their share of (inclusive) reproduction within the bounds of certain constraints (Johnstone, 2000; Buston *et al.*, 2009). Thus, this proposed mechanism for the emergence of groups with only some breeders does not satisfy the third condition of my definition. Simply put, the optima of reproductive skew models are driven almost entirely by conflict (Queller and Strassmann, 2018). Consequently, the conditions that should lead to high reproductive skew are those in which one party may best outcompete, fight, or control the other, which will generally not be the conditions that favour reproductive division of labour as defined here.

Nevertheless, reproductive skew theory is a large, alternative body of work, relying on a different causal emphasis, but with much overlap and providing its own set of predictions and explanations for many of the same patterns in nature to which my work is intended to apply. For instance, reproductive skew predictions have been used to explain patterns in eusocial insects and cooperative breeders (Reeve and Keller, 2001). Which body of evolutionary theory can best explain observed patterns in nature will reveal whether conflict or cooperation is the more dominant force in shaping complex biological societies.

Summary of thesis

In my work, I have developed evolutionary theory to try to understand the factors that influence the evolution of division of labour and its various forms. In particular, I have focused on reproductive division of labour, involving only one cooperative trait and between individuals of the same species. As such, my work is most relevant to understanding the role played by division of labour in the fraternal major transitions, such as those to multicellularity and obligate eusociality (Queller, 2000).

I was particularly interested in the effect of genetic relatedness (Grafen, 1985). Since cooperation is a defining feature of division of labour (condition 2), relatedness may play an important role in the evolution of these kinds of behaviours. Indeed, when individuals within a group are more likely to share genes by common descent (high relatedness), this can foster the evolution of cooperation as genes may spread both directly through the offspring of their bearer or indirectly through the offspring of neighbouring relatives who share the gene (Hamilton, 1964). Accordingly, empirical work has found that transitions to multicellularity and eusociality have only ever occurred in social groups of maximal relatedness (clonal or singly-mated respectively) (Hughes *et al.*, 2008; Boomsma, 2009; Fisher, Cornwallis and West, 2013). A key question of my thesis is whether maximal relatedness (no conflict) is a necessary condition for the evolution of extreme division of labour.

In chapter 2, I develop a model to investigate the factors that favour the evolution of division of labour and the conditions that may lead to an extreme specialisation between sterile helpers and pure reproductives. This work was published in *Nature Ecology and Evolution* and the published manuscript in its journal format is also presented in the supplementary materials (Cooper and West, 2018).

In chapter 3, I developed a series of simple models to determine the kinds of cooperative systems that can lead to the evolution of a majority of sterile helpers within labour dividing groups. This was motivated by a result of chapter 2 and other theoretical works, predicting that the number of sterile helpers could never stably exceed the number of purely reproductive individuals.

In chapter 4, I investigate the factors that influence the mechanism whereby cooperating individuals specialise into either sterile helper or purely reproductive roles. In particular, I develop a model to determine whether individuals should specialise randomly, or employ environmental information to do so in a coordinated fashion.

In much of my work (chapters 2 and 3), I employ open models to examine the effect of relatedness on predicted evolutionary outcomes (Gardner and West, 2006). An open model treats relatedness as an independently varying parameter. In contrast, closed models make demographic and life history assumptions such that relatedness varies as a function of other model parameters (such as dispersal rates or matedness). In chapter 5, I present a perspective that I published in *Evolution Letters* with Samuel Levin, Geoff Wild and Stuart West that examines the empirical success of open and closed models and provides guidelines on when one or the other approach should be used. The published manuscript is presented in its journal format in the supplementary materials (Cooper *et al.*, 2018).

In chapter 6, I summarize the key results of my work and discuss ways in which my research may be extended to address three key questions for the evolution of division of labour and its impact on biological complexity.

References

Alberts, B. *et al.* (2008) *Molecular Biology of the Cell*, *The Yale Journal of Biology and Medicine*. New York: Garland. doi: 10.1024/0301-1526.32.1.54.

Boomsma, J. J. (2009) 'Lifetime monogamy and the evolution of eusociality', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, pp. 3191–3207. doi: 10.1098/rstb.2009.0101.

Boraas, M. E., Seale, D. B. and Boxhorn, J. E. (1998) 'Phagotrophy by flagellate selects for colonial prey: A possible origin of multicellularity', *Evolutionary Ecology*, 12, pp. 152–164. doi: 10.1023/A:1006527528063.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Buston, P. M. *et al.* (2009) 'Reproductive skew and the evolution of conflict resolution : a synthesis of transactional and tug-of-war models', *Behavioral Ecology*, 20, pp. 672–684. doi: 10.1093/beheco/arp050.

Cooper, G. A. *et al.* (2018) 'Modelling relatedness and demography in social evolution', *Evolution Letters*, 2, pp. 260–271.

Cooper, G. A. and West, S. A. (2018) 'Division of labour and the evolution of extreme specialization', *Nature Ecology and Evolution*. Springer US, 2, pp. 1161–1167. doi: 10.1038/s41559-018-0564-9.

Davies, N. B. and Brooke, M. de L. (1988) 'Cuckoos versus reed warblers: Adaptations and counteradaptations', *Animal Behaviour*, 36, pp. 262–284. doi: 10.1016/S0003-3472(88)80269-0.

Davies, N. B., Krebs, J. R. and West, S. A. (2012) *An Introduction to Behavioural Ecology*, *An introduction to behavioural ecology*. Hoboken, New Jersey: John Wiley & Sons. doi: 10.1037/026600.

Dawkins, R. (1976) *The selfish gene*, *The selfish gene*. Oxford, UK: Oxford University Press. doi: 10.2307/2065678.

Fisher, R. M., Cornwallis, C. K. and West, S. A. (2013) 'Group formation, relatedness, and the evolution of multicellularity', *Current Biology*, 23, pp. 1120–1125. doi: 10.1016/j.cub.2013.05.004.

Gardner, A. and Grafen, A. (2009) 'Capturing the superorganism: A formal theory of group adaptation', *Journal of Evolutionary Biology*, 22, pp. 659–671. doi: 10.1111/j.1420-9101.2008.01681.x.

Gardner, A. and West, S. A. (2006) 'Demography, altruism, and the benefits of budding', *Journal of Evolutionary Biology*, 19, pp. 1707–1716. doi: 10.1111/j.1420-9101.2006.01104.x.

Grafen, A. (1985) 'A geometric view of relatedness', *Oxford Surveys in Evolutionary Biology*, 2, pp. 28–89.

Grafen, A. (1999) 'Formal Darwinism, the individual-as-maximizing-agent analogy and bet-hedging', *Proceedings of the Royal Society B: Biological Sciences*, 266, pp. 799–803. doi: 10.1098/rspb.1999.0708.

Hamilton, W. D. (1964) 'The genetical evolution of social behaviour I-II', *Journal of Theoretical Biology*, 7, pp. 1–52. doi: 10.1016/0022-5193(64)90038-4.

Hughes, W. O. H. *et al.* (2008) 'Ancestral monogamy shows kin selection is key to the evolution of eusociality', *Science*, 320, pp. 1213–1216. doi: 10.1126/science.1156108.

Johnstone, R. A. (2000) 'Models of reproductive skew: A review and synthesis', *Ethology*, 106, pp. 5–26. doi: 10.1046/j.1439-0310.2000.00529.x.

Johnstone, R. A. and Cant, M. A. (2010) 'The evolution of menopause in cetaceans and

humans: The role of demography', *Proceedings of the Royal Society B: Biological Sciences*, 277, pp. 3765–3771. doi: 10.1098/rspb.2010.0988.
Maynard Smith, J. (1976) 'Group Selection', *Quarterly Review of Biology*, 51, p. 277.
Oster, G. F. and Wilson, E. O. (1978) *Caste and ecology in the social insects*, *Caste and ecology in the social insects*. Princeton: Princeton University Press.
Queller, D. C. (1997) 'Cooperators since life began', *Q Rev Bio*, 72, pp. 184–188.
Queller, D. C. (2000) 'Relatedness and the fraternal major transitions', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 355, pp. 1647–1655. doi: 10.1098/rstb.2000.0727.
Queller, D. C. and Strassmann, J. E. (2018) 'Evolutionary Conflict', *Annual Review of Ecology, Evolution, and Systematics*, 49, pp. 73–93.
Reeve, H. R. and Keller, L. (2001) 'Tests of reproductive skew models', *Annual review of entomology*, 46, pp. 347–385.
Seeley, T. D. (2014) *Honeybee ecology: a study of adaptation in social life.*, *Monographs in behavior and ecology*. Princeton: Princeton University Press. doi: 10.1016/0169-5347(86)90077-7.
Smith, A. (1776) *An Inquiry into the Nature and Causes of the Wealth of Nations*. 6 (1950). London: Methuen.
Strassmann, J. E., Zhu, Y. and Queller, D. C. (2000) 'Altruism and social cheating in the social amoeba Dictyostelium discoideum', *Nature*, 408, pp. 965–967. doi: 10.1038/35050087.
Szathmáry, E. and Maynard Smith, J. (1995) *The major evolutionary transitions*, *Nature*. Oxford: Oxford University Press. doi: 10.1038/374227a0.
Tortora, G. J. and Derrickson, B. (2014) *Principles of Anatomy and Physiology*, *Wiley*. United States of America: John Wiley & Sons. doi: 10.1016/S0031-9406(05)60992-3.
Vehrencamp, S. L. (1979) 'The Roles of Individual, Kin, and Group Selection in the Evolution of Sociality', in *Social behavior and communication*. Boston, MA: Spriner, pp. 351–394.
West, S. A. *et al.* (2015) 'Major evolutionary transitions in individuality', *Proceedings of the National Academy of Sciences*, 112, pp. 10112–10119. doi: 10.1073/pnas.1421402112.
West, S. A. and Cooper, G. A. (2016) 'Division of labour in microorganisms: An evolutionary perspective', *Nature Reviews Microbiology*, 14, pp. 716–723. doi: 10.1038/nrmicro.2016.111.
West, S. A., Griffin, A. S. and Gardner, A. (2007) 'Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection', *Journal of Evolutionary Biology*, 20, pp. 415–432. doi: 10.1111/j.1420-9101.2006.01258.x.

Chapter 2: Division of labour and the evolution of extreme specialisation[*]

Abstract

Division of labour is a common feature of social groups, from biofilms to complex animal societies. However, we lack a theoretical framework that can explain why division of labour has evolved on certain branches of the tree of life but not others. We model the reproductive division of labour over one cooperative behaviour, considering both when it should evolve and the extent to which the different types should become specialised. We found that: (1) reproductive division of labour is usually—but not always—favoured by high efficiency benefits to specialisation and low within-group conflict; and (2) natural selection favours extreme specialisation, where some individuals are completely dependent upon the helping behaviour of others. We make a number of predictions, several of which are supported by the existing empirical data, from microbes and animals, while others suggest novel directions for empirical work. More generally, we show how reproductive division of labour can lead to mutual dependence between different individuals and hence drive major evolutionary transitions, such as those to multicellularity and eusociality.

Introduction

Division of labour is a defining feature of complexity at all levels of biological organization (Szathmáry and Maynard Smith, 1995; Queller, 2000; Boomsma, 2007; Bourke, 2011; West *et al.*, 2015) If individuals specialise to perform certain tasks, more complex social groups can evolve. In the extreme, if the different individuals become

[*] Published as Cooper, G. A., and West, S. A. (2018) Division of labour and the evolution of extreme specialisation. *Nature Ecology and Evolution*. 2, 1161–1167 (appended).

dependent upon the tasks performed by others, then a new `higher level' individual may emerge. Examples include genes with different functions in a genome, cells that form distinct tissues in an animal, and castes that carry out different tasks in social insect societies. Consequently, in order to understand why complex life has evolved, we must understand the evolution of division of labour.

We lack theory that can explain why division of labour has evolved on some branches of the tree of life, but not others. Previous work has focused on clonal groups of cells and eusocial insects (Oster and Wilson, 1978; Wilson, 1978; Michod, 2006; Tannenbaum, 2007; Willensdorfer, 2009; Rossetti *et al.*, 2010; Ispolatov, Ackermann and Doebeli, 2012; Rueffler, Hermisson and Wagner, 2012; Solari, Kessler and Goldstein, 2013). In both of these cases, it has often been assumed that the fitness interests of individuals are perfectly aligned, and so the evolution of division of labour is favoured if it increases group fitness (supplementary tables 1 & 2). However, division of labour also arises in species such as bacteria, slime moulds and cooperatively breeding animals where there can be appreciable conflict within groups and so cannot be assumed to be `superorganisms' (Arnold, Owens and Goldizen, 2005; Bourke, 2011; West and Cooper, 2016). If there is conflict within groups, then division of labour would not be selected for just because it increases group fitness (Hamilton, 1964; Ackermann *et al.*, 2008; Gardner and Grafen, 2009). Furthermore, if division of labour plays a role in driving transitions such as those to multicellularity and eusociality, then we need to understand how it can first evolve from individual level selection (Michod, 1997; Bourke, 2011; West *et al.*, 2015).

Division of labour can take different forms (**Figure 1**). In the simplest possible scenario, with only one cooperative behaviour, reproductive division of labour consists of `helpers'

and `reproductives' that may be specialized to varying degrees. The helper could be a fully specialised, sterile helper or a generalist that both helps and reproduces. Similarly, the more reproductive type could be a pure reproductive or a generalist that engages in some helping. This suggests four broad types of division of labour—from two different generalist types that help and reproduce at different rates, to the extreme case of a sterile helper paired with a pure reproductive (**Figure 1**). However, most models assume that only a certain type of division of labour is possible, often with fully specialised sterile helpers (Oster and Wilson, 1978; Ackermann *et al.*, 2008; Willensdorfer, 2009; Rossetti *et al.*, 2010; Solari, Kessler and Goldstein, 2013). (supplementary tables 1 & 2). Therefore, these models cannot be used to explain variation in the form that division of labour takes.

We theoretically model how a number of factors could influence selection for reproductive division of labour and its various forms. We wish to find the conditions that would favour both the initial evolution of such division of labour, and the evolution of extreme specialisation, with individuals losing the ability to reproduce independently. We are interested in insights that could be applied across a range of different biological systems. Consequently, we construct a deliberately simple approximation, focusing on the trade-offs that we hypothesise are likely to be of general importance, rather than a complex model of a specific system (Frank, 1998).

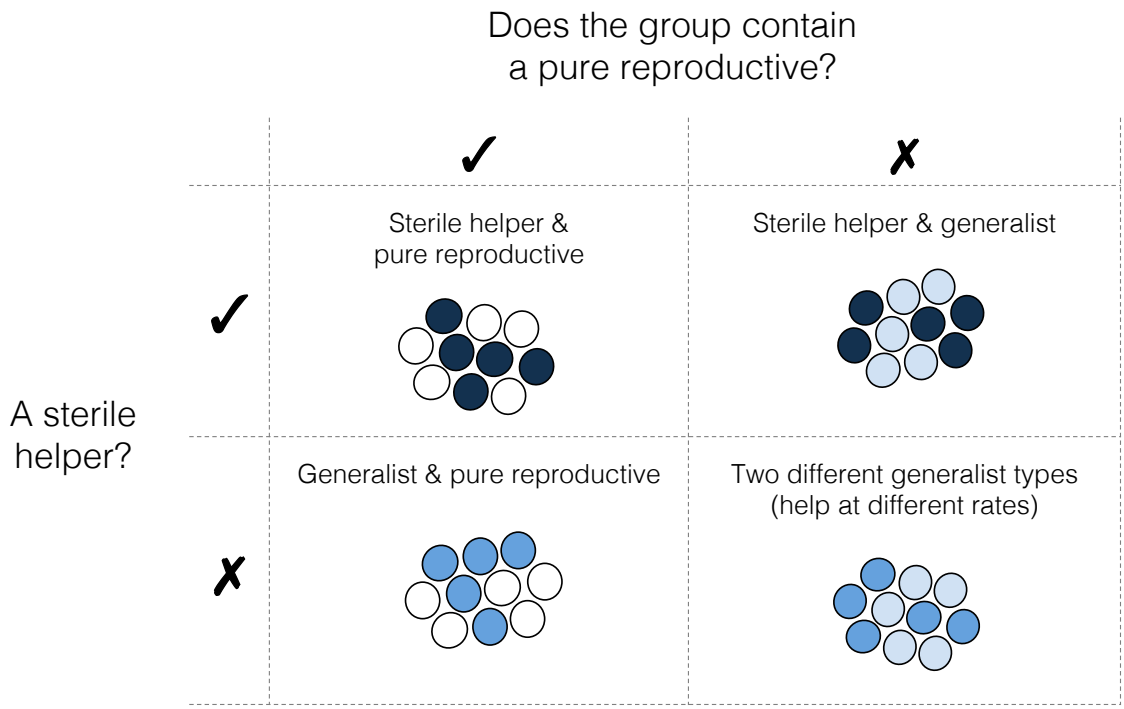


Figure 1: the different possible forms of reproductive division of labour. There are four broad forms of reproductive division of labour, each defined by the presence or absence of the two fully specialised phenotypes: pure reproductives (i.e. germline cells or social insect queens) and sterile helpers (i.e. somatic cells or worker castes). A *sterile helper and pure reproductive* division of labour (top left) is composed of both fully specialised phenotypes. The three other kinds of division of labour contain at least one generalist phenotype that invests in both tasks. In the strategy containing two different generalist phenotypes (bottom right), one of these phenotypes is cooperating at a higher level than the other

Model

We consider an infinite population that is divided into social groups of fixed, finite sizes in which individuals engage in social interactions locally but offspring compete globally for niches in the next generation (island model). The genetic relatedness between individuals in a social group is given by R, which represents the relative probability that they are genotypically identical by common descent (see appendix).

We allow individuals to perform a costly cooperative behaviour, which increases the survival or reproductive viability of social group members. Specifically, a fraction $1 - \lambda$

of the benefit of cooperation goes to the focal individual and the remaining fraction λ is distributed to the other members of the social group (**Figure 2c**). We allow for potential efficiency benefits from greater cooperation with the parameter α (**Figure 2b**), which determines whether the benefits from increased cooperation are linear ($\alpha = 1$), accelerating ($\alpha > 1$) or decelerating ($\alpha < 1$). We vary the extent to which the cooperative trait is essential for survival with the parameter e. If $e = 1$, then cooperation is essential and individuals that reside in social groups with no cooperation have a fitness of zero. As e decreases, the trait is less essential and the cooperative behaviour becomes more of a luxury activity.

We allow for a reproductive division of labour into two phenotypes (**Figure 2a**). At the start of their life cycles, individuals terminally adopt phenotype 1 with probability p and phenotype 2 with probability $1 - p$. Phenotype 1 invests a fraction q_1 of its lifetime efforts toward the cooperative trait and the remaining fraction $1 - q_1$ is allocated toward personal survival or reproduction. In contrast, phenotype 2 invests q_2 into the cooperative trait. When the two phenotypes differ, we will tend to assume that $q_1 > q_2$, such that, without loss of generality, phenotype 1 is more cooperative. Consequently, we are allowing three independent traits to co-evolve in our model: the level of cooperation of each phenotype (q_1, q_2), and the relative ratio of the two phenotypes (p). In our analysis, we used equilibrium theory to determine the strategy that is expected to evolve in the long-term (see appendix and supplementary information 1, 2 & 3) (Parker and Maynard Smith, 1990; Frank, 1998). The key predictions of our model are given in Table 1.

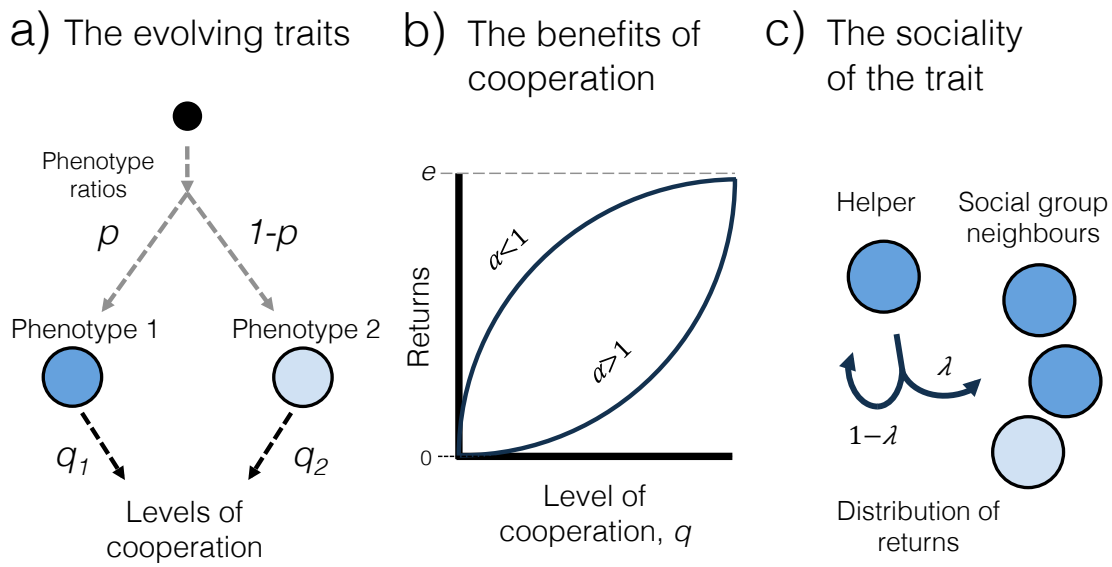


Figure 2: a reproductive division of labour model. We consider a cooperative trait that individuals may invest in at a private cost. (a) The evolving traits. At the start of its life-cycle, an undifferentiated individual (top, shaded) stochastically adopts one of two phenotypes (middle). Individuals become phenotype 1 with probability p and phenotype 2 otherwise. Each phenotype invests a fixed amount of lifetime effort (q_1 or q_2) into the cooperative trait (bottom). When the two phenotypes differ, we tend to assume that phenotype 1 invests more into cooperation ($q_1 > q_2$). The traits p, q_1 and q_2 are the characteristics that are allowed to evolve in the model. (b) The benefits of cooperation. We allow for accelerating ($\alpha > 1$) or diminishing ($\alpha < 1$) returns to increased investment in cooperation. Each fecund individual has a baseline benefit $1 - e$ regardless of the social environment. If $e = 1$, the trait is essential. Otherwise ($e < 1$), it is non-essential. The maximal return that can be attained via cooperation is then given by e (trait essentiality). (c) The trait sociality (or shareability). A proportion λ of the returns from personal investment in cooperation will benefit social group neighbours equally (others-only; focal helper excluded.) The remaining $1 - \lambda$ benefits the focal helper alone. An additional parameter, R (not shown), quantifies the degree of relatedness within social groups of the population (others-only).

What types of division of labour are stable?

Our model allows several possible strategies: uniform non-cooperation (no individuals help), uniform cooperation (all individuals are identical generalists that both help and reproduce) and four different types of division of labour (**Figure 1**). The types of division of labour are defined by the presence or absence of the extreme possible phenotypes: sterile helpers ($q_1 = 1$) and pure reproductives ($q_2 = 0$) (**Figure 1**). We found that

uniform non-cooperation, uniform cooperation and division of labour could all arise as long-term evolutionary strategies (**Figure 3**).

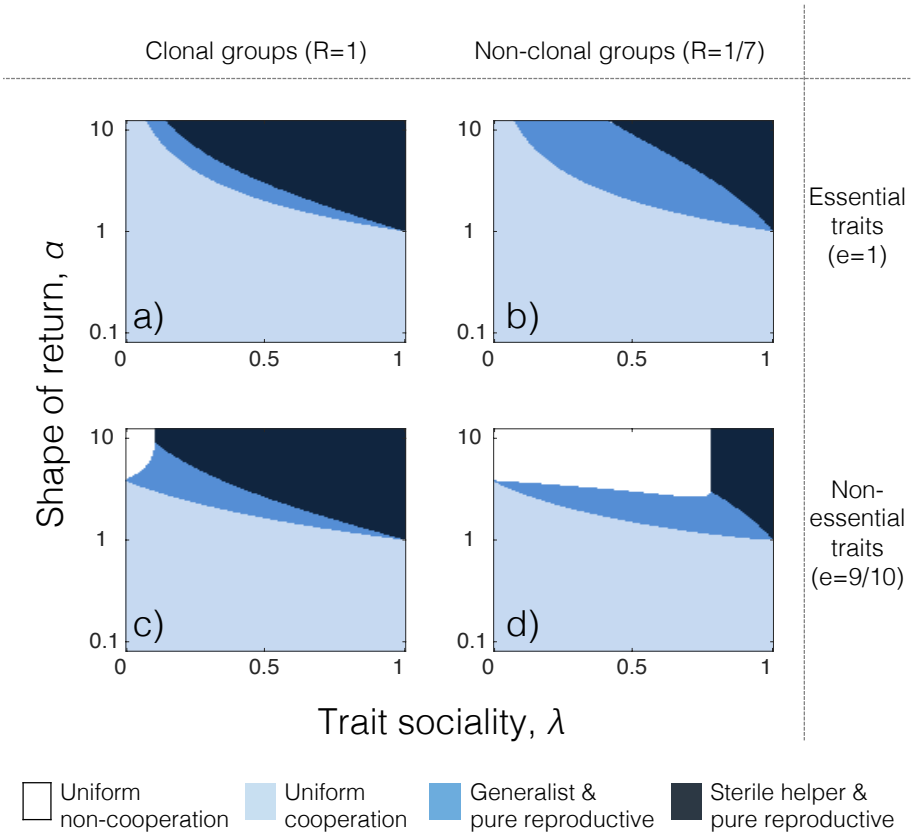


Figure 3: the evolution of reproductive division of labour. We examine how the various factors influence the strategy that is expected to evolve. Intermediate division of labour composed of generalists and pure reproductives is broadly favoured by increasing benefits to specialisation, high trait sociality, high trait essentiality and high relatedness. If these factors are pushed to further extremes, then an extreme form of division of labour with sterile helpers and pure reproductives may be favoured. No other form of division of labour is observed to be stable. See supplementary information 1-3 for more details.

We found that there was an evolutionary bias to more extreme forms of reproductive division of labour, where one of the phenotypes does all of the cooperation (**Figure 3**). The two types of division of labour that could be favoured were those with a pure reproductive ($q_2 = 0$) paired with either a generalist helper ($0 < q_1 < 1$) or with a sterile helper ($q_1 = 1$). In contrast, we did not find a region of parameter space where either of

the other two types of division of labour, in which both phenotypes engage in cooperation, could evolve (**Figure 3**). Specifically, the combination of a generalist ($0 < q_2 < 1$) with either a more cooperative generalist ($q_2 < q_1 < 1$) or with a sterile helper ($q_1 = 1$) was never found to be stable. In supplementary information 4, we show that these results hold if we relax the assumption that cooperative costs are linear.

Why are intermediate forms of reproductive division of labour, where both phenotypes cooperate, not stable? We hypothesise that there may be an evolutionary feedback loop in which helper specialisation drives reproductives to help less and reproductive specialisation drives helpers to help more. In order to test this hypothesis, we developed dynamic, individual-based simulations as a proof of principle (**Figure 4**; supplementary information 5).

We held the level of cooperation in one phenotype fixed (q_1 or q_2) and allowed the other phenotype to evolve. We found that when phenotype 2 invested more resources into reproduction, phenotype 1 invested more resources into cooperation (lower q_2 drives higher q_1; **Figure 4a**). In turn, when phenotype 1 invested more resources into cooperation, phenotype 2 was driven more rapidly to pure reproduction ($q_2 = 0$; **Figure 4b**). More generally, the higher we fixed the level of cooperation of one phenotype, the higher the investment into reproduction of the other phenotype (**Figure 4c**).

To examine how these effects feedback on to each other, we considered the consequences of allowing just one phenotype to evolve for some time and before allowing both phenotypes to evolve. We initially held fixed the level of cooperation of phenotype 2 ($0 < q_2$ fixed), which lead to the other phenotype evolving to an intermediate level of

cooperation ($q_1 < 1$; **Figure 4d**). When we then allowed both phenotypes to evolve, they always drove each other to the specialist extremes of pure reproduction ($q_2 = 0$) and sterile helping ($q_1 = 1$; **Figure 4d**).

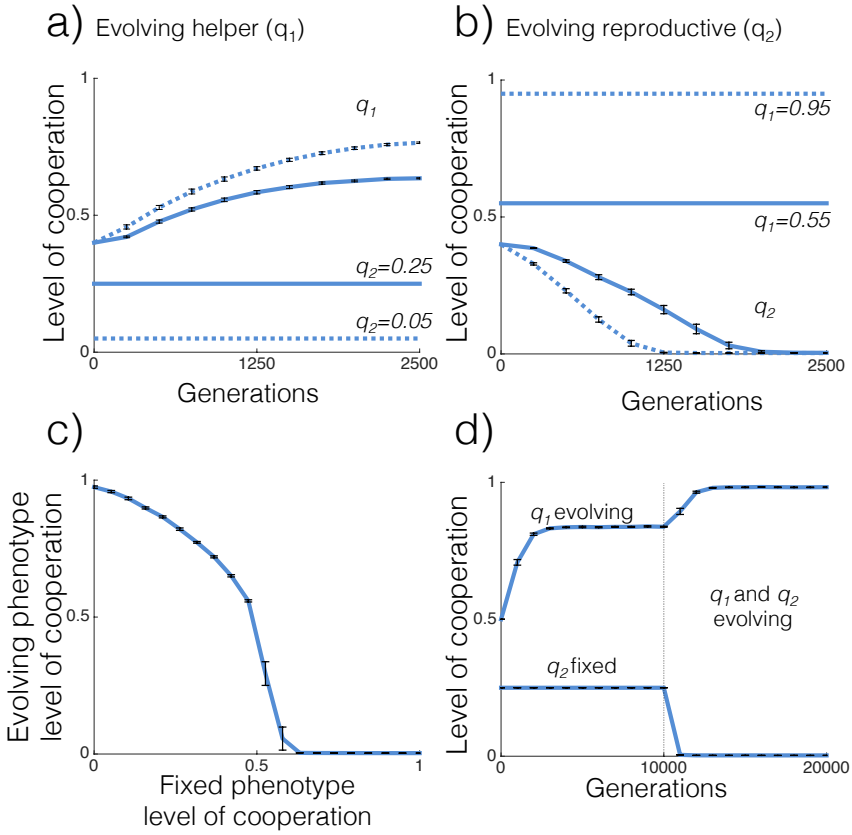


Figure 4: the evolution of extreme specialisation. We hypothesized that there exists an evolutionary feedback loop whereby helper specialisation drives reproductives to specialise further on reproduction and reproductive specialisation drives helpers to help more. As a proof of principle, we tested this hypothesis with dynamic, individual-based simulations. (a) We find that the level of cooperation of the helper phenotype, q_1, evolves to higher levels of cooperation when the reproductive phenotype, q_2, is more fully specialised (low q_2; dashed) than when it is less specialised (high q_2; solid). (b) We find that the level of cooperation of the reproductive phenotype, q_2, evolves more quickly to pure reproduction ($q_2 = 0$) when the helper phenotype, q_1, is more fully specialised (high q_1; dashed) than when it is less specialised (low q_1; solid). (c) If we hold one phenotype fixed we find that the lower the level of cooperation of the fixed phenotype, the higher the level of cooperation of the evolving phenotype and vice versa. (d) If we hold reproductive specialisation (q_2) fixed for 10,000 generations, then the level of helper cooperation (q_1) evolves stably to an intermediate value. If we then allow the level of cooperation of both phenotypes (q_1 and q_2) to evolve for another 10,000 generations, then both phenotypes are driven to their specialised extremes ($q_1 = 1$ and $q_2 = 0$.) All error bars are 95 percent confidence intervals over simulation repetitions. See supplementary information 5 for more details.

The only intermediate form of reproductive division of labour that we find to be stable is the pairing of a generalist with a pure reproductive ($0 < q_1 < 1;\ q_2 = 0$). In a later section, we discuss how one of the conditions required for division of labour to be favoured is that there are efficiency benefits to specialisation ($\alpha > 1$). If division arises, we also found that the same condition ($\alpha > 1$) always favours the stability of pure reproduction ($q_2 = 0$). Consequently, whenever division of labour evolves, one phenotype will always be a pure reproductive ($q_2 = 0$). In contrast, efficiency benefits to specialisation ($\alpha > 1$) are necessary but *not* sufficient for the stability of a sterile helper ($q_1 = 1$). The evolution of a sterile helper therefore requires more restrictive conditions then a pure reproductive and thus an intermediate division of labour composed of the former phenotype but not the latter would never occur ($q_1 = 1;\ q_2 > 0$).

Reproductive division of labour in nature

Our prediction that more extreme forms of reproductive division of labour should be observed correspond to the patterns observed in the natural world. Considering cell groups, the most common form of division appears to be between sterile helpers and pure reproductives (Fisher, Cornwallis and West, 2013; West and Cooper, 2016). One of the clearest examples is the germ-soma divide in multicellular animals. Similarly, in microorganisms such as bacteria, fungi, algae and slime moulds, there are numerous examples of a sterile helper paired with a pure reproductive (Strassmann, Zhu and Queller, 2000; Velicer, Kroos and Lenski, 2000; Ackermann *et al.*, 2008; Herron *et al.*, 2009; Flores and Herrero, 2010). In contrast, less extreme division of labour involving a generalist paired with either a pure reproductive or a sterile helper appears to be relatively rare, with a single example of each from bacteria and algae respectively (Herron and Michod, 2008; Veening *et al.*, 2008).

In animal groups, there appears to be two most common forms of reproductive division of labour. First, in the social insects, the divide between queens and their workers is between pure reproductive and sterile or effectively sterile-workers (Oster and Wilson, 1978; Boomsma, 2007; Bourke, 2011). Second, in cooperative breeding vertebrates and invertebrates, the division is commonly associated with age—individuals help when young, and breed when old (Arnold, Owens and Goldizen, 2005; Bourke, 2011). While our model captures the essence of why division of labour is favoured for these species, they also introduce a number of other factors, such as costs and benefits of cooperation varying with age, relatedness asymmetries, and individuals who are `failed breeders' (Koenig and Dickinson, 2004; Arnold, Owens and Goldizen, 2005). However, as predicted by our model, there are no known instances of division of labour in animals between a sterile helper and a generalist (that engages both in breeding and in helping others breed).

Our examination of the pattern in nature requires two points of clarification. First, in all these cases, the appropriate comparison is one trait at a time. So, pure reproductives with respect to one trait, may engage in other cooperative behaviours. For example, in the cyanobacterial division of labour, the cells that do not fix nitrogen are pure reproductives with respect to that trait, but can perform other cooperative traits, such as photosynthesis (Flores and Herrero, 2010). Second, there may be an observation bias towards discovering more extreme division of labour. Our prediction emphasizes the need for a quantitative survey of the types of division of labour in nature, rather than a reliance on just the systems that are being studied.

Our results do not categorically forbid the other two other types of reproductive division of labour in nature. Instead, our analysis offers a simple null model such that, if a form of division of labour not predicted by our model has evolved, then there must exist a complexity in the biological system not captured by our model and its assumptions. An example is provided by division of labour between sterile helpers and generalists in some Volvocine algae lineages (Herron and Michod, 2008). When these algae reproduce, the reproductive cells must grow to the size of offspring colonies before reproduction. As such, any reproduction comes with a large commitment of resources, leading to helper sterility providing a large discontinuous resource bonus not contained in our model (Michod, 2006).

Relatedness and division of labour

A standard assumption has been that a higher relatedness favours reproductive division of labour (Bourke, 2011; Fisher, Cornwallis and West, 2013; West and Cooper, 2016). Indeed, many models of division of labour have assumed the extreme relatedness of clonality, or that it is group fitness that is being maximized (Oster and Wilson, 1978; Michod, 2006; Willensdorfer, 2009; Rossetti *et al.*, 2010; Solari, Kessler and Goldstein, 2013). In contrast, we found that relatedness (R) has no influence on whether reproductive division of labour is favoured for essential traits ($e = 1$) that are required for reproduction or survival (**Figure 3a-3b**). A higher relatedness has no influence because the fitness benefit of being a pure reproductive is then exactly cancelled by the indirect fitness cost of not helping relatives in the group.

However, for non-essential traits ($e < 1$), a higher relatedness (higher R) does favour the evolution of reproductive division of labour (**Figure 3c-3d**). The main reason for this is

that, as relatedness decreases, the indirect benefits of cooperation are reduced, and so uniform non-cooperation can outcompete cooperative division of labour (Ackermann *et al.*, 2008). Combining our trends, the overall prediction is that a higher relatedness (higher R) will favour division of labour for some traits (non-essential; $e < 1$), but not for other traits (essential; $e = 1$). The extent to which a trait is essential may change over evolutionary time—for example, a trait might start as relatively non-essential, and then become more essential as a group becomes more social, with more division of labour. In this case, relatedness could be more important for the initial evolution of division of labour than for its later maintenance. An empirical example is the subsequent loss of strict lifetime monogamy in some eusocial insects (Hughes *et al.*, 2008).

In the empirical data from multicellular groups, a higher relatedness is correlated with a greater likelihood of reproductive division of labour (Fisher, Cornwallis and West, 2013). This is consistent with our model if the data are drawn only from non-essential traits, or a mix of essential and non-essential traits. In animal groups, a higher relatedness, due to lower levels of promiscuity, also leads to individuals being more likely to spend time as a helper in cooperative breeding vertebrates (Cornwallis *et al.*, 2010; Lukas and Clutton-Brock, 2012). Our predictions suggest that it would be useful to further divide traits on the basis of how essential they are, and then test for how this interacts with relatedness.

Considering the different types of reproductive division of labour, our model predicts that a higher relatedness (higher R) favours more extreme division of labour regardless of whether the trait is essential or non-essential ($0 < e \leq 1$; **Figure 3**). In particular, a higher relatedness favours division between a sterile helper and pure reproductive ($q_1 = 1$, $q_2 = 0$) over division between a generalist and a pure reproductive ($0 < q_1 < 1$, $q_2 = 0$).

Our predicted influence of relatedness is consistent with the empirical data for multicellular groups, where groups with a higher relatedness are more likely to have sterile helpers (Fisher, Cornwallis and West, 2013). Experimental evolution studies have also found that the sterile helpers are disfavoured at relatively low relatedness, in both slime moulds and fungi (Kuzdzal-Fick *et al.*, 2011; Bastiaans, Debets and Aanen, 2016). In animal groups, the division between sterile helper and pure reproductive also appears to be favoured by a higher relatedness, with eusociality having only evolved in sexual species that have strict lifetime monogamy, or asexual species that reproduce clonally (Giron *et al.*, 2004; Hughes *et al.*, 2008; Boomsma, 2009).

In contrast to our predictions and the empirical data, some have argued that monogamy (higher R) may sometimes disfavour cooperation and division of labour (Nonacs, 2011; Olejarz *et al.*, 2015). However, subsequent work showed that these conclusions are based on restrictive assumptions. For example, in Nonacs's model, the best way for individuals to `help' relatives is to disperse and reduce competition for resources rather than to stay and help kin (Nonacs, 2011; Leggett *et al.*, 2012). In turn, the results of Olejarz et al.'s model are an artefact of constraining the analysis to the invasion of unconditional sterility in colonies where only an intermediate proportion of sterile workers is optimal (supplementary information 7.5) (Olejarz *et al.*, 2015; Davies and Gardner, 2018).

Clonal groups and lifetime monogamy

While higher relatedness tends to favour division of labour, our model shows that maximal relatedness ($R = 1$) is not required for reproductive division of labour to evolve, or even for the most extreme form of division between sterile helpers and pure reproductives

($q_1 = 1$, $q_2 = 0$; **Figure 3**) (Ackermann *et al.*, 2008). Many previous models of division of labour have assumed maximal relatnedness ($R = 1$), such that there is no conflict within-groups, and analysed how division of labour can maximise group fitness (Oster and Wilson, 1978; Michod, 2006; Willensdorfer, 2009; Ispolatov, Ackermann and Doebeli, 2012; Solari, Kessler and Goldstein, 2013). We have shown that division of labour can still be favoured, even with relatively low relatedness ($R < 1$) where there can be appreciable within-group conflict. This is consistent with Hamilton's rule, which showed how altruistic sterile helping can be favoured when $R < 1$ (Hamilton, 1964; Ackermann *et al.*, 2008). More generally, this emphasises how division of labour can be favoured by kin selection at the level of the individual rather than simply by group efficiency maximisation.

Our prediction that maximal relatedness is not necessary is supported by cases where division of labour with sterile and reproductive helpers has been observed in non-clonal multicellular groups (Strassmann, Zhu and Queller, 2000; Fisher, Cornwallis and West, 2013). In social insects, lifetime monogamy leads to a potential helper being equally related to their siblings and their own offspring, which is equivalent to $R = 1$ in our asexual model (Boomsma, 2007; Hughes *et al.*, 2008; Gardner and Grafen, 2009; Fisher, Cornwallis and West, 2013). Consequently, although eusociality has only evolved in species with lifetime monogamy or asexual reproduction, our theory shows that the initial evolution of division of labour, while favoured by maximal relatedness, does not require this condition in principle.

Ecological benefits and further predictions. Many previous models found that division of labour is favoured when there is an efficiency benefit to specialisation, with non-linear returns to increased cooperation ($\alpha > 1$) (Michod, 2006, 2007; Ispolatov, Ackermann and

Doebeli, 2012; Solari, Kessler and Goldstein, 2013). In supplementary information 7.1, we show that an efficiency benefit to specialisation ($\alpha > 1$) is necessary, but not sufficient for the evolution of reproductive division of labour (**Figure 3**) (Willensdorfer, 2009; Gavrilets, 2010). Instead our model also makes a suite of predictions for how the efficiency benefits of increased cooperation interact with a number of other factors (Table 1; supplementary information 7.2 & 7.3). For example, division of labour is more likely to evolve if the benefits of cooperation are generously shared between individuals (high λ), and if the trait is very essential for survival (high e).

Our model also makes predictions about the factors that favour the most extreme form of division of labour, with sterile helpers and pure reproductives (high α, λ, e and R), and the factors that determine the optimal ratio of helpers to reproductives, (p^*) (Table 1; supplementary information 7.4). These different factors can interact in unforeseen ways that qualitatively change predictions. For example, whether an increase in efficiency benefit of specialisation (α) and trait sociality (λ) leads to higher, lower or has no influence on the optimal proportion of helpers (p^*) can depend on the type of division of labour that is favoured (**Figure 5**).

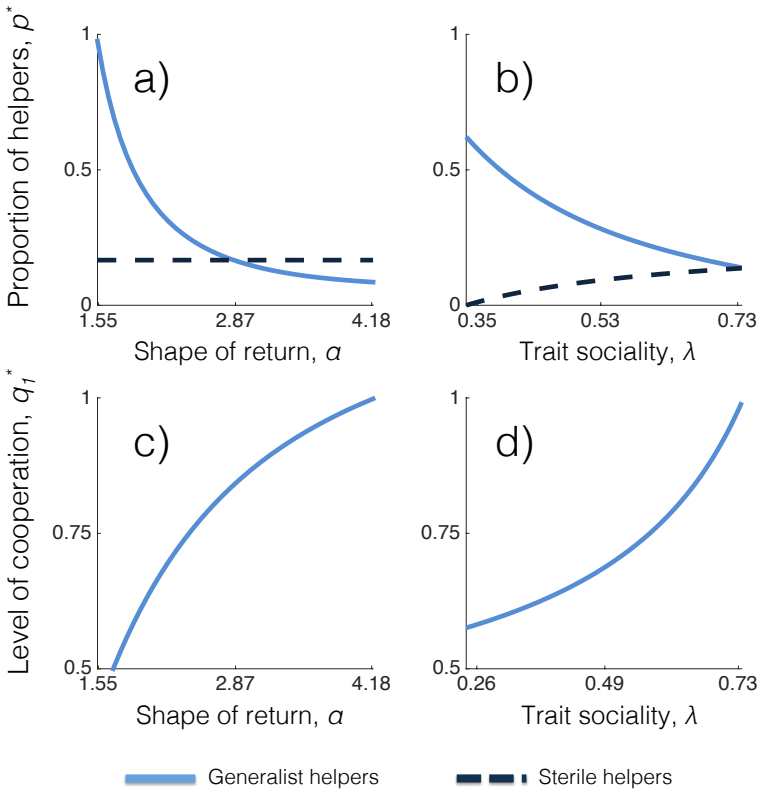


Figure 5: the proportion of helpers and the level of cooperation. Our model makes a number of predictions about what form reproductive division of labour should take, including the optimal proportion of helpers and their level of cooperation. We found that the way certain factors affect the proportion of helpers depends on the form of division of labour that is favoured. For example, (a) if the helpers are sterile ($q_1^* = 1$), then an increase in the efficiency benefits of specialisation (higher α) has no effect upon the optimal proportion (p^*) of helpers ($q_1^* = 1$). However, if the helpers are generalists ($0 < q_1^* < 1$), then a higher α decreases the optimal proportion of helpers (lower p^*). Similarly, (b) if the helpers are sterile ($q_1^* = 1$), then an increase in the sociality of the trait (higher λ) increases the optimal proportion (higher p^* of helpers ($q_1^* = 1$). However, if the helpers are generalists ($0 < q_1^* < 1$) then a higher λ decreases the optimal proportion of helpers (lower p^*). These different predictions arise because, when there are generalists, the amount that they help (q_1^*) also changes (c and d). So for example, with a high efficiency benefit (higher α we predict few generalists (lower p^*; a) but who help a lot (high q_1^*; c). In contrast, an increase in social group relatedness (higher R) or trait essentiality (higher e) leads to an increase in the optimal helper proportion (higher p^*) regardless of the form of division that is favoured ($0 < q_1^* \leq 1$; not shown). See supplementary information 7.4 for more details.

Life-history and population demography

As we are interested in patterns that hold across a range of different biological systems, we constructed a deliberately simple model, focusing on the factors that we believe are likely to be of broad importance (see appendix). For example, we purposefully left relatedness as

an independent parameter (`open' model), and assumed that competition for breeding spots was global (Frank, 1998). In some cases, for specific species, or groups of species, the way that the demographic processes generate relatedness patterns may be important for the evolution of division of labour. For these cases, our predictions may not hold and it could be useful to develop `closed' models to examine how relatedness is determined by population demography and to make more targeted predictions (Lehmann and Rousset, 2010). We solve a closed model in supplementary information 6 and show that limited dispersal and overlapping generations both lead to higher relatedness in a way that favours the evolution of sterile helper and pure reproductive division of labour over uniform non-cooperation.

Broadly, our conceptual understanding of division of labour has been anchored to a limited number of complex systems, particularly the eusocial insects, cooperative breeders, and certain obligate multicellular organisms. Our model did not incorporate a number of factors that have been argued to be important in these systems, such as haplodiploid genetics, partially overlapping generations and large group sizes (Hamilton, 1964; Seger, 1983; Bonner, 2004; Michod, 2006; Bourke, 2011; Quiñones and Pen, 2017). Furthermore, we did not restrict our model to the extreme case of maximal group relatedness, with clonal groups formed from single cells (or family groups from lifetime monogamy). Instead, our results show that the evolution of reproductive division of labour does not require such specific life-history characteristics and can evolve in much simpler cases. More generally, there is a rich precedent in evolutionary theory of using the predictions of simple models to better understand the behaviour of complex systems (Parker and Maynard Smith, 1990; Frank, 1998; Bourke, 2011).

Conclusion

To conclude, we found that when reproductive division of labour is favoured, it tends to adopt extreme forms, involving pure reproductives that are dependent upon the helping behaviour of others. We found that helper sterility may evolve even with appreciable within-group conflict. This illustrates that division of labour is not merely a group level adaptation that evolves to maximise group efficiency (Gardner and Grafen, 2009). Reproductive division of labour can be favoured by kin selection at the level of the individual and play a significant role in members of social groups becoming dependent upon each other. Consequently, division of labour is a driver, not a consequence, of major evolutionary transitions to higher levels of individuality, such as multicellularity and eusociality.

Appendix 1: the fitness equation.

We write the fitness of an individual as its expected fitness averaged across the possible phenotypes. Specifically, the neighbour-modulated (direct) fitness of a focal mutant with strategy (p, q_1, q_2) is given by:

$$W = p(1-q_1)((1-e) + e((1-\lambda)q_1^{\alpha} + \lambda(PQ_1^{\alpha} + (1-P)Q_2^{\alpha})$$
$$+ (1-p)(1-q_2)((1-e) + e((1-\lambda)q_2^{\alpha} + \lambda(PQ_1^{\alpha} + (1-P)Q_2^{\alpha}) \quad \text{(A1)}$$

where P, Q_1 and Q_2 are the average, others-only trait-values of social group neighbours (Taylor and Frank, 1996; Frank, 1998; Brown and Taylor, 2010). The two terms (top row, bottom row) represent the realised fitness when of phenotype 1 (with probability p) and phenotype 2 (with probability $1-p$) respectively. Alternatively, the fitness equation may be conceptualized as the fitness of a founding individual of a social group, expressed as an expectation over the fitness of its descendants in the last generation of the social group before dispersal (haystack model.) The essentiality of the trait, e, is defined as the fraction of the realised fitness benefit that arises from cooperation rather than the asocial environment. The fitness benefit from cooperation in turn is composed of the benefit from personal investment in cooperation $(1-\lambda)(\ldots)$ and the benefit that arises from the investment of social group neighbours $\lambda(\ldots)$. The benefit due to cooperation of social group neighbours is equal to $\sum_{i=1}^{N}(p_i q_{1,i}^{\alpha} + (1-p_i)q_{2,i}^{\alpha})/N$where i is an index of social group members that does not include the focal individual. We approximate this as $PQ_1^{\alpha} + (1-P)Q_2^{\alpha}$, which holds under rare mutation and weak selection (arithmetic mean is approximately equal to the geometric mean in this case.)

Appendix 2: Equilibrium analysis

We seek the Evolutionarily Stable Strategy (ESS), (p^*, q_1^*, q_2^*), which is the strategy that, when employed by all individuals in the population, is uninvadable by a rare mutant lineage with an alternate strategy (Parker and Maynard Smith, 1990). In supplementary information 1, we use numerical methods to determine the equilibria of the model, except in a number of special cases where we are able to solve for the equilibria analytically. An equilibrium point is defined as a joint strategy (p, q_1, q_2) for which directional selection in each trait is zero. We employ the directional selection forms developed by Taylor and Frank and Brown and Taylor (Taylor and Frank, 1996; Brown and Taylor, 2010). For example, directional selection in p is given as $W_p(p, q_1, q_2) = {\partial W}/{\partial p} + R\,{\partial W}/{\partial P}$, where the partial derivative are evaluated for a monomorphic population ($p = P$, $q_1 = Q_1$, $q_2 = Q_2$) and R is the relatedness of interacting individuals. We employ an open model approach and assume that R is a fixed, independent parameter of the model, and that it adopts the same value for all traits. An equilibrium strategy is then an ESS if it is uninvadable such that rare mutants are always less fit than an arbitrary individual in the equilibrium population. In supplementary information 2, we confirm that the equilibria of our model are uninvadable, and hence ESSs, with an analytical uninvadability analysis, numerical verification and individual-based simulations. In supplementary information 3, we use the methodology of Brown and Taylor to show that all of the ESSs analysed are convergent stable, such that the population is expected to evolve toward the equilibrium in trait-space (Brown and Taylor, 2010).

Appendix 3: Model assumptions

The construction of our model and its analysis relies on a set of life history, demographic and evolutionary assumptions, each of which may limit the applicability of the model in

specific cases, for specific species. For example, we assumed that the population is infinite, structured into groups of fixed size, that reproduction is asexual with non-overlapping generations and that mutations are rare and lead to weak differences in selection. We also assumed that all competition is global. Taken as a whole, this constructs a model for reproductive division of labour that is only exact for very simple forms of life and we do not claim that our model makes exact predictions for division of labour in all species. However, we contend that our predictions should also hold broadly in nature when averaged across the tree of life. This will be true so long as our assumptions have not removed or rendered rigid a factor that is consistently important for the evolution of reproductive division of labour.

In some cases, factors that we have not modelled may be subsumed into the analysis. For example, although our model does not explicitly model the role of group size (N) in the evolution of division of labour, such predictions may be generated if we assume a relationship between group size and the other factors in our model. For example, in the Volvocine algae, it has been argued that the efficiency benefit of specialisation (α) is an increasing function of group size such that $\alpha = \alpha(N)$ and $\alpha'(N) > 0$. In this case, assuming that cooperation is favoured, we recover the previously found result that increasing group size N favours division of labour (Michod *et al.*, 2006). Alternatively, if the benefits of cooperation are shared less equally in larger groups (lower λ) then larger groups would disfavour division of labour.

In supplementary tables 1 and 2, we summarize how our model compares and links to previous theoretical work on the evolution of reproductive division of labour.

	Model predictions	Empirical validation?
When is divison of labour favoured?	1. (a) If the trait is non-essential $e < 1$, higher relatedness (higher R) favours division of labour. (b) If the trait is essential ($e = 1$), there is no effect of the value of relatedness.	Clonal cell groups ($R = 1$) are more likely to have a division of labour (Fisher, Cornwallis and West, 2013). In animal groups, lower levels of promiscuity (higher R), leads to individuals being more likely to spend time as a helper in cooperative breeding vertebrates (Cornwallis *et al.*, 2010; Lukas and Clutton-Brock, 2012). In all cases, distinction between essential and non-essential traits is not tested.
	2. (a) If relatedness, trait sociality and trait essentiality are high (higher R, λ and e), a higher efficiency benefit to specialisation (higher e) favours division of labour. (b) Otherwise, uniform non-cooperation may be favoured.	Formal test needed. However, greater group size does correlate with division of labour in some systems and this may be due to altered efficiency benefits (Michod, 2006; Herron and Michod, 2008; Herron *et al.*, 2009).
	3. Higher trait sociality (higher λ) favours division of labour.	-
	4. If relatedness and trait sociality are low and the efficiency benefits are high (low R and λ; high α), a higher trait essentiality (higher e) favours division of labour.	-
	5. Depending on how group size (N) influences factors such as the efficiency benefits to specialisation (α), the extent to which the benefits of cooperation are shared (λ) or social group relatedness (R), a larger group may favour or disfavour division of labour.	Larger colony sizes have been found to favour division of labour in the Volvocine algae (Michod, 2006).
What kind of division is favoured?	6. The only forms of division that are favoured are those with a pure reproductive ($q_2 = 0$) paired with either a sterile helper ($q_1 = 1$) or a helper-reproductive ($0 < q_1 < 1$).	Formal test needed. Of the 7 discussed examples of microbial division of labour, 5 are sterile helper and pure reproductive, 1 is generalist and pure reproductive and 1 is sterile helper and generalist division of labour (Strassmann, Zhu and Queller, 2000; Velicer, Kroos and Lenski, 2000; Herron and Michod, 2008; Veening *et al.*, 2008; Herron *et al.*, 2009; Diard *et al.*, 2013)
When are sterile helpers favoured?	7. Higher relatedness (higher R) favours helper sterility.	Clonal cell groups ($R = 1$) are more likely to have sterile cells (Fisher, Cornwallis and West, 2013). Sterile helpers are disfavoured at low relatedness in both slime moulds and fungi (Kuzdzal-Fick *et al.*, 2011; Bastiaans, Debets and Aanen, 2016). In animal groups, eusociality has only evolved under conditions of strict lifetime monogamy (higher R) (Giron *et al.*, 2004; Boomsma, 2007; Hughes *et al.*, 2008).
	8. (a) If relatedness, trait essentiality and trait sociality are high (high R, e and λ), higher	-

	efficiency benefits to specialisation (higher α) favours helper sterility. (b) Otherwise, higher efficiency benefits (higher α) may favour uniform non-cooperation.	
	9. Higher trait sociality (higher λ) favours helper sterility.	-
	10. If relatedness and trait sociality are low and the efficiency benefits are high (low R and λ; high α), higher trait essentiality (higher e) favours helper sterility.	-
What affects the proportion of helpers?	11. Higher relatedness (higher R) favours a higher proportion of helpers (higher p^*).	Clonal cell groups ($R = 1$) have a higher proportion of helpers but study lacks phylogenetically independent comparisons and so is not statistically significant (more data needed)
	12. (a) If the helpers are sterile ($q_1 = 1$), a higher efficiency benefits to specialisation (higher α) favours a lower proportion of helpers (lower p^*). (b) Otherwise, there is no effect.	-
	13 If helpers are sterile $q_1 = 1$, higher trait sociality (higher λ) favours a higher proportion of helpers (higher p^*) (b) Otherwise, higher trait sociality (higher λ) favours a lower proportion of helpers (lower p^*).	-
	14. Higher trait essentiality (higher e) favours a higher proportion of helpers (higher p^*).	-

Table 1: model predictions and empirical validation. We present the key predictions of our model with respect to the conditions in which reproductive division of labour is favoured, what kind of division may be favoured, whether the extreme form of division with sterile helpers and pure reproductives is favoured and the factors that affect the proportion of helpers (if division is favoured.) We also specify whether the predictions have been previously tested empirically. The entry `-' implies that an empirical test is needed.

References

Ackermann, M. *et al.* (2008) 'Self-destructive cooperation mediated by phenotypic noise', *Nature*, 454, pp. 987–990. doi: 10.1038/nature07067.

Arnold, K. E., Owens, I. P. F. and Goldizen, A. W. (2005) 'Division of labour within cooperatively breeding groups', *Behaviour*, 142, pp. 1577–1590. doi: 10.1163/156853905774831927.

Bastiaans, E., Debets, A. J. M. and Aanen, D. K. (2016) 'Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters', *Nature Communications*, 7, pp. 1–10. doi: 10.1038/ncomms11435.

Bonner, J. T. (2004) 'Perspective: The size-complexity rule', *Evolution*, 58, pp. 1883–1890. doi: 10.1111/j.0014-3820.2004.tb00476.x.

Boomsma, J. J. (2007) 'Kin Selection versus Sexual Selection: Why the Ends Do Not Meet', *Current Biology*, 17, pp. R672–R683. doi: 10.1016/j.cub.2007.06.033.

Boomsma, J. J. (2009) 'Lifetime monogamy and the evolution of eusociality', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, pp. 3191–3207. doi: 10.1098/rstb.2009.0101.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Brown, S. P. and Taylor, P. D. (2010) 'Joint evolution of multiple social traits: a kin selection analysis.', *Proceedings. Biological sciences / The Royal Society*, 277, pp. 415–422. doi: 10.1098/rspb.2009.1480.

Cornwallis, C. K. *et al.* (2010) 'Promiscuity and the evolutionary transition to complex societies', *Nature*, 466, pp. 969–972. doi: 10.1038/nature09335.

Davies, N. G. and Gardner, A. (2018) 'Monogamy promotes altruistic sterility in insect societies', *Royal Society Open Science*, 5, p. 172190. doi: 10.1098/rsos.172190.

Diard, M. *et al.* (2013) 'Stabilization of cooperative virulence by the expression of an avirulent phenotype', *Nature*, 494, pp. 353–356. doi: 10.1038/nature11913.

Fisher, R. M., Cornwallis, C. K. and West, S. A. (2013) 'Group formation, relatedness, and the evolution of multicellularity', *Current Biology*, 23, pp. 1120–1125. doi: 10.1016/j.cub.2013.05.004.

Flores, E. and Herrero, A. (2010) 'Compartmentalized function through cell differentiation in filamentous cyanobacteria', *Nature Reviews Microbiology*, 8, pp. 39–50. doi: 10.1038/nrmicro2242.

Frank, S. A. (1998) *Foundations of Social Evolution*, *Monographs in behavior and ecology*. Princeton: Princeton University Press. doi: 10.1038/sj.hdy.6885351.

Gardner, A. and Grafen, A. (2009) 'Capturing the superorganism: A formal theory of group adaptation', *Journal of Evolutionary Biology*, 22, pp. 659–671. doi: 10.1111/j.1420-9101.2008.01681.x.

Gavrilets, S. (2010) 'Rapid transition towards the division of labor via evolution of developmental plasticity', *PLoS Computational Biology*, 6, p. e1000805. doi: 10.1371/journal.pcbi.1000805.

Giron, D. *et al.* (2004) 'Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition', *Nature*, 430, p. 676. doi: 10.1038/nature02721.

Hamilton, W. D. (1964) 'The genetical evolution of social behaviour I-II', *Journal of Theoretical Biology*, 7, pp. 1–52. doi: 10.1016/0022-5193(64)90038-4.

Herron, M. D. *et al.* (2009) 'Triassic origin and early radiation of multicellular volvocine algae', *Proceedings of the National Academy of Sciences*, 106, pp. 3254–3258. doi: 10.1073/pnas.0811205106.

Herron, M. D. and Michod, R. E. (2008) 'Evolution of complexity in the volvocine algae: Transitions in individuality through Darwin's eye', *Evolution*, 62, pp. 436–451. doi: 10.1111/j.1558-5646.2007.00304.x.

Hughes, W. O. H. *et al.* (2008) 'Ancestral monogamy shows kin selection is key to the evolution of eusociality', *Science*, 320, pp. 1213–1216. doi: 10.1126/science.1156108.

Ispolatov, I., Ackermann, M. and Doebeli, M. (2012) 'Division of labour and the evolution of multicellularity', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 1768–1776. doi: 10.1098/rspb.2011.1999.

Koenig, W. D. and Dickinson, J. L. (2004) *Ecology and evolution of cooperative breeding in birds*, *Ecology and Evolution of Cooperative Breeding in Birds*. Cambridge: Cambridge University Press.

Kuzdzal-Fick, J. J. *et al.* (2011) 'High relatedness is necessary and sufficient to maintain multicellularity in Dictyostelium', *Science*, 334, pp. 1548–1551. doi: 10.1126/science.1213272.

Leggett, H. C. *et al.* (2012) 'Promiscuity and the evolution of cooperative breeding.', *Proceedings. Biological sciences / The Royal Society*, 279, pp. 1405–1411. doi: 10.1098/rspb.2011.1627.

Lehmann, L. and Rousset, F. (2010) 'How life history and demography promote or inhibit the evolution of helping behaviours', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, pp. 2599–2617. doi: 10.1098/rstb.2010.0138.

Lukas, D. and Clutton-Brock, T. H. (2012) 'Cooperative breeding and monogamy in mammalian societies', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 2151–2156. doi: 10.1098/rspb.2011.2468.

Michod, R. E. (1997) 'Evolution of the Individual', *The American Naturalist*, 150, pp. s5–s21. doi: 10.1086/286047.

Michod, R. E. *et al.* (2006) 'Life-history evolution and the origin of multicellularity', *Journal of Theoretical Biology*, 239, pp. 257–272. doi: 10.1016/j.jtbi.2005.08.043.

Michod, R. E. (2006) 'The group covariance effect and fitness trade-offs during evolutionary transitions in individuality', *Proceedings of the National Academy of Sciences*, 103, pp. 9113–9117. doi: 10.1073/pnas.0601080103.

Michod, R. E. (2007) 'Evolution of individuality during the transition from unicellular to multicellular life', *Proceedings of the National Academy of Sciences*, 104, pp. 8613–8618. doi: 10.1073/pnas.0701489104.

Nonacs, P. (2011) 'Monogamy and high relatedness do not preferentially favor the evolution of cooperation', *BMC Evolutionary Biology*, 11, p. 58. doi: 10.1186/1471-2148-11-58.

Olejarz, J. W. *et al.* (2015) 'The evolution of non-reproductive workers in insect colonies with haplodiploid genetics', *eLife*, 4, p. e08918. doi: 10.7554/eLife.08918.

Oster, G. F. and Wilson, E. O. (1978) *Caste and ecology in the social insects*, *Caste and ecology in the social insects*. Princeton: Princeton University Press.

Parker, G. A. and Maynard Smith, J. (1990) 'Optimality theory in evolutionary biology', *Nature*, 348, pp. 27–33. doi: 10.1038/348027a0.

Queller, D. C. (2000) 'Relatedness and the fraternal major transitions', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 355, pp. 1647–1655. doi: 10.1098/rstb.2000.0727.

Quiñones, A. E. and Pen, I. (2017) 'A unified model of Hymenopteran preadaptations that trigger the evolutionary transition to eusociality', *Nature Communications*, 8, p. 15920. doi: 10.1038/ncomms15920.

Rossetti, V. *et al.* (2010) 'The evolutionary path to terminal differentiation and division of

labor in cyanobacteria', *Journal of Theoretical Biology*, 262, pp. 23–24. doi: 10.1016/j.jtbi.2009.09.009.
Rueffler, C., Hermisson, J. and Wagner, G. P. (2012) 'Evolution of functional specialization and division of labor.', *Proceedings of the National Academy of Sciences of the United States of America*, 109, pp. e326–e335. doi: 10.1073/pnas.1110521109.
Seger, J. (1983) 'Partial bivoltinism may cause alternating sex-ratio biases that favour eusociality', *Nature*, 301, pp. 59–62. doi: 10.1038/301059a0.
Solari, C. A., Kessler, J. O. and Goldstein, R. E. (2013) 'A General Allometric and Life-History Model for Cellular Differentiation in the Transition to Multicellularity', *The American Naturalist*, 181, pp. 369–380. doi: 10.1086/669151.
Strassmann, J. E., Zhu, Y. and Queller, D. C. (2000) 'Altruism and social cheating in the social amoeba Dictyostelium discoideum', *Nature*, 408, pp. 965–967. doi: 10.1038/35050087.
Szathmáry, E. and Maynard Smith, J. (1995) *The major evolutionary transitions*, *Nature*. Oxford: Oxford University Press. doi: 10.1038/374227a0.
Tannenbaum, E. (2007) 'When does division of labor lead to increased system output?', *Journal of Theoretical Biology*, 247, pp. 413–425. doi: 10.1016/j.jtbi.2007.03.020.
Taylor, P. D. and Frank, S. A. (1996) 'How to make a kin selection model', *Journal of Theoretical Biology*, 180, pp. 27–37. doi: 10.1006/jtbi.1996.0075.
Veening, J.-W. *et al.* (2008) 'Transient heterogeneity in extracellular protease production by Bacillus subtilis', *Molecular Systems Biology*, 4, p. 184. doi: 10.1038/msb.2008.18.
Velicer, G. J., Kroos, L. and Lenski, R. E. (2000) 'Developmental cheating in the social bacterium Myxococcus xanthus', *Nature*, 404, pp. 598–601. doi: 10.1038/35007066.
West, S. A. *et al.* (2015) 'Major evolutionary transitions in individuality', *Proceedings of the National Academy of Sciences*, 112, pp. 10112–10119. doi: 10.1073/pnas.1421402112.
West, S. A. and Cooper, G. A. (2016) 'Division of labour in microorganisms: An evolutionary perspective', *Nature Reviews Microbiology*, 14, pp. 716–723. doi: 10.1038/nrmicro.2016.111.
Willensdorfer, M. (2009) 'On the evolution of differentiated multicellularity', *Evolution*, 63, pp. 306–323. doi: 10.1111/j.1558-5646.2008.00541.x.
Wilson, E. O. (1978) 'The ergonomics of caste in the social insects', *American Economic Review*, 102, pp. 41–66. doi: 10.1086/282522.

Supplementary information

This supplementary information contains further analysis and results. In section 1, we derive the equilibrium conditions that were used to show when each of the possible strategies was the ESS in our analysis. This includes separate conditions for the two different generalist types (section 1.1), for generalist and pure reproductive division of labour (section 1.2), for sterile helper and generalist division of labour (section 1.3), for sterile helper and pure reproductive division of labour (section 1.4) as well as for uniform non-cooperation (section 1.5) and uniform cooperation (section 1.6). In sections 2 and 3, we show that these equilibria are indeed uninvadable and convergent stable and thus ESSs of the model. In our model we assumed that the cost of cooperation is linear with respect to helper investment. In section 4, we show that our results hold if the cost of cooperation is allowed to be non-linear. In section 5, we test our hypothesis that extreme forms of specialisation are favoured by an evolutionary feedback loop between the levels of cooperation of each phenotype via dynamic, individual-based simulations. We did not specify the demographic processes that generate relatedness in our model and instead assumed that R was an independent parameter (an 'open' model). In section 6, we 'close' a simplification of our model by including limited dispersal and overlapping generations as processes that lead to the build-up of genetic correlations between interacting individuals. In section 7, we discuss further predictions of our model. This includes further discussion of how the efficiency benefits of specialisation (section 7.1), the sociality of the trait (section 7.2) and the essentiality of the trait (section 7.3) favour division of labour. We also further discuss predictions for the optimal proportion of helpers in a division of labour (section 7.4) and how our results compare to a previous, population genetic model of sterility in haplodiploid insects (section 7.5). In supplementary tables 1 and 2, we summarise how our model compares and links to previous theoretical work on the evolution of division of labour.

1 Supplementary information: equilibrium analysis

We discretised parameter space and checked, for all combinations of parameter values, whether each of the possible strategies was an equilibrium of the system. We considered R={1 (clonal), 1/3 (non-clonal; not presented) 1/7 (non-clonal)}, e={1 (essential), 9/10 (non-essenital), 8/10 (non-essential; not presented)}, $\log(\alpha)$= $\{-2.5, \ldots, 2.5\}$ and $\lambda = \{1/100, \ldots, 1\}$. The discretisations of $\log(\alpha)$ and λ were each evenly spaced over 151 nodes. The results of this analysis are depicted in Figure 3.

Here, we derive the conditions that were used to show when each of the possible strategies of the model are equilibria of the fitness landscape.

An *equilibrium* strategy is a fixed-point of the system: natural selection within a monomorphic population, to a first order-approximation, does not act to 'push' a mutant strain in any permissible direction locally. An equilibrium strategy is *convergent stable* if it is an attractor of the dynamical system: a monomorphic population close to the equilibrium strategy always experiences directional selection that favours a rare mutant lineage with trait value closer to the equilibrium[9]. An equilibrium is *uninvadable* if is a local maximum of the fitness landscape[43]. If this is the case, we say that the equilibrium is an Evolutionarily Stable Strategy (ESS), where an ESS is the joint strategy employed by all cells in the population such that no mutant lineage with an alternate strategy can successfully invade the population[25,26]. We expect the ESS to be the long-term strategy that a system will evolve to.

In subsequent sections, we show how convergence stability and uninvadability were evaluated. In particular, we found that all equilibria considered by our numerical discretization were convergent stable and uninvadable. As such, which strategy is an ESS of a system is determined entirely by the equilibrium conditions of the model. Overall, we found that one and only one strategy was the ESS for all parameter combinations except those for which $\alpha = 1$ and $\lambda = 1$, where there was no ESS.

Let the fitness of an arbitrary individual from a rare mutant strain be given by:

$$W = p(1-q_1)\Big[(1-e)+e\big((1-\lambda)q_1^{\alpha}+\lambda(PQ_1^{\alpha}+(1-P)Q_2^{\alpha})\big)\Big] + (1-p)(1-q_2)\Big[(1-e)+e\big((1-\lambda)q_2^{\alpha}+\lambda(PQ_1^{\alpha}+(1-P)Q_2^{\alpha})\big)\Big].$$

Let $W_p(p,q_1,q_2) = {}^{\partial W}/_{\partial p} + R{}^{\partial W}/_{\partial P}$, $W_{q_1}(p,q_1,q_2) = {}^{\partial W}/_{\partial q_1} + R{}^{\partial W}/_{\partial Q_1}$ and $W_{q_2}(p,q_1,q_2) = {}^{\partial W}/_{\partial q_2} + R{}^{\partial W}/_{\partial Q_2}$ be the respective directional selection terms for p, q_1 and q_2, where all partial derivatives are evaluated at $p = P, q_1 = Q_1, q_2 = Q_2$ [5,45]. These are given here:

$$\begin{aligned} W_p = \quad & (1-q_1)\big[(1-e)+e\big((1-\lambda)q_1^{\alpha}+\lambda(pq_1^{\alpha}+(1-p)q_2^{\alpha})\big)\big] \\ & -(1-q_2)\big[(1-e)+e\big((1-\lambda)q_2^{\alpha}+\lambda(pq_1^{\alpha}+(1-p)q_2^{\alpha})\big)\big] \\ & +p(1-q_1)e\lambda(q_1^{\alpha}-q_2^{\alpha})R \\ & +(1-p)(1-q_2)e\lambda(q_1^{\alpha}-q_2^{\alpha})R \end{aligned}$$

$$\begin{aligned} W_{q_1} = \quad & -p\big[(1-e)+e\big((1-\lambda)q_1^{\alpha}+\lambda(pq_1^{\alpha}+(1-p)q_2^{\alpha})\big)\big] \\ & +p(1-q_1)e(1-\lambda)\alpha q_1^{\alpha-1} \\ & +p(1-q_1)e\lambda p\alpha q_1^{\alpha-1}R \\ & +(1-p)(1-q_2)e\lambda p\alpha q_1^{\alpha-1}R \end{aligned}$$

$$
\begin{aligned}
W_{q_2} \quad &= -(1-p)\big[(1-e)+e\big((1-\lambda)q_2^{\alpha}+\lambda(pq_1^{\alpha}+(1-p)q_2^{\alpha})\big)\big] \\
&\quad +(1-p)(1-q_2)e(1-\lambda)\alpha q_2^{\alpha-1} \\
&\quad +p(1-q_1)e\lambda(1-p)\alpha q_2^{\alpha-1}R \\
&\quad +(1-p)(1-q_2)e\lambda(1-p)\alpha q_2^{\alpha-1}R
\end{aligned}
$$

For each trait, directional selection quantifies the direction in which a rare mutant with a slight change in the trait value may increase its fitness when in a population that is otherwise monomorphic for all three trait values (increase in trait value favoured if directional selection is positive and decrease in trait value favoured if directional selection is negative).

In the following, we solve for the equilibrium conditions of each strategy in turn.

1.1 Two different generalist types

Division of labour with two different generalist types occurs in the interior of the state-space, in the domain: $\mathbf{D1} = (0 < p < 1, 0 < q_1 < 1, 0 < q_2 < 0; q_1 \neq q_2)$. The last condition states that the cooperative investment of each phenotype must be distinct, otherwise the strategy corresponds to uniform cooperation. We employ the classic method developed by Taylor and Frank and Brown and Taylor in this case[5,45]. We solve first for the equilibrium value (p^*, q_1^*, q_2^*) such that directional selection in each trait is zero: $W_p(p^*, q_1^*, q_2^*) = W_{q_1}(p^*, q_1^*, q_2^*) = W_{q_2}(p^*, q_1^*, q_2^*)$=0. We do this by solving for the null-planes as a function of (q_1, q_2) for each trait and finding the joint value (q_1^*, q_2^*) for which the three planes intersect. This is done numerically for the full model. If we assume that the cooperative trait is essential ($e = 1$) and others-only ($\lambda = 1$), then we have the

following nullplanes:

$$q_1\text{-nullplane:} \qquad p(q_1, q_2) = \frac{q_2^\alpha - (1-q_2)\alpha R q_1^{\alpha-1}}{q_2^\alpha - q_1^\alpha + \alpha R q_1^{\alpha-1}(q_2 - q_1)}$$

$$q_2\text{-nullplane:} \qquad p(q_1, q_2) = \frac{q_2^\alpha - (1-q_2)\alpha R q_2^{\alpha-1}}{q_2^\alpha - q_1^\alpha + \alpha R q_2^{\alpha-1}(q_2 - q_1)}$$

By setting these equal and rearranging, we arrive at the equation:

$$\alpha = \frac{\log(1-q_1) - \log(1-q_2)}{\log(q_1) - \log(q_2)}$$

If $q_1 > q_2$ then the numerator of the above equation is negative, the denominator is positive, and thus $\alpha < 0$ which is not permitted in our model. The same result holds if we stipulate that $q_2 > q_1$. We conclude that there are no intersections of the two nullplanes in the simplified model.

In the full model, we solved numerically for an intersection point of the three nullplanes and found no such point within the state space for any of the considered parameter combinations. As such, we conclude that two different generalist types is never a form of division of labour that is an ESS in our analysis.

1.2 Generalist and pure reproductive division of labour

Generalist and pure reproductive division of labour occurs in the symmetrically equivalent domains $\mathbf{D2} = (0 < p < 1, 0 < q_1 < 1, q_2 = 0)$ or $\mathbf{D3} = (0 < p < 1, q_1 = 0, 0 < q_2 < 1)$. Without loss of generality let us consider only the domain **D2**. For this strategy, one of the traits, q_2, is now a boundary trait ($q_2 = 0$; in contrast to an interior trait: $0 < q_2 < 1$) and as such the methodology for evaluating whether the strategy is an equilibrium with respect to q_2 must be adapted. There are two sources of instability for this strategy: loss of partial division of labour and loss of pure reproduction.

1.2.1 Stability to loss of partial division of labour

If we consider only the interior traits, p and q_1, then the analysis proceeds with the approach developed by Brown and Taylor[5]. We first solve for the nullclines of p and q_1 as functions of q_1. We then solve for the equilibrium values (p^*, q_1^*) such that the nullclines intersect (no directional selection in either trait). We do this numerically for the full model. If no such strategy, (p^*, q_1^*), exists then we conclude that generalist and pure reproductive division of labour is not an ESS. For the simplified model in which the trait is essential ($e = 1$), we have the following nullclines:

$$p\text{-nullcline:} \qquad p(q_1) = \frac{\lambda R + (1-\lambda)(1-q_1)}{\lambda(R+1)} \frac{1}{q_1}$$

$$q_1\text{-nullcline:} \qquad p(q_1) = \frac{\alpha(\lambda R + (1-\lambda)) - q_1(1-\lambda)(1+\alpha)}{\lambda(\alpha R + 1)} \frac{1}{q_1}$$

These are monotonically decreasing functions from positive infinity at $q_1 = 0$. At $q_1 = 1$, the p-nullcline is equal to $R/(R+1)$, which is strictly contained in $]0, 1[$ and the q_1-nullcline is strictly less than 1. Thus, both nullclines traverse the domain $[0, 1] \times [0, 1]$. Directional selection in both traits is negative at the point, $(p = 1, q_1 = 1)$. If $\alpha < 1$ the nullclines do not intersect in the positive quadrant and the q_1 nullcline is strictly less than the p-nullcline for all value of $0 \leq q_1 \leq 1$ and thus the equilibrium strategy is the intersection point of the q_1-nullcline with the boundary $p = 1$ (uniform cooperation; under the constraint that $q_2 = 0$). If $\alpha > 1$, then the nullclines intersect in the positive quadrant (q_1 nullcline crosses p-nullcline from above) and the equilibrium strategy (ignoring directional selection in q_2) depends on where the intersection point of the nullclines occurs. If the nullclines intersect before entering the domain, $(0 \leq p \leq 1, 0 \leq q_1 \leq 1)$, then the equillibrium is once again the intersection point of the q_1-nullcline with the boundary $p = 1$ (uniform cooperation; under the constraint that $q_2 = 0$). If the nullclines intersect after departing the domain $q_1^* > 1$, then the equilibrium strategy is the intersection point of the p-nullcline with the upper boundary of $q_1 = 1$ (sterile helper and pure reproductive division of labour; under

the constraint that $q_2 = 0$). By exclusion, we have that generalist and pure reproductive is the equilibrium strategy (under the constraint that $q_2 = 0$) if the nullclines intersect within the domain.

We can determine whether this is the outcome by considering directional selection at the aforementioned intersection points of each nullcline with the upper boundary of the opposing trait. The q_1-nullcline intersects the upper boundary, $p = 1$, at $q_1^* = \alpha(\lambda R + (1-\lambda))/(1 + \alpha(\lambda R + (1-\lambda))$. If $W_p(1, q_1^*, 0) = (1-q^*)q^{*\alpha} - \lambda q^{*\alpha} + (1-q^*)\lambda R q^{*\alpha} < 0$, then the intersection point of the nullclines occurs after they have entered the domain. Meanwhile, the p-nullcline intersects the upper boundary, $q_1 = 1$, at $p^* = R/(R+1)$. If $W_{q_1}(p^*, 1, 0) = -p^*((1 - \lambda + p^*\lambda) + (1-p^*)\alpha\lambda R p^* < 0$, then the intersection point of the nullclines occurs before exiting the domain. If the first condition is not satisfied then the equilibrium strategy is uniform cooperation under the constraint that $q_2 = 0$. If the second condition is not satisfied then the equilibrium strategy is sterile helper and pure reproductive under the constraint that $q_2 = 0$. In contrast, generalist and pure reproductive is stable to loss of partial division of labour mutations if both constraints are satisfied, giving the broader condition: $(1/\lambda < \alpha < (R + (1-\lambda))/\lambda R)$. If we further assume that $\lambda = 1$, then we find that these constraints collapse both to 1 and that thus that generalist and pure reproductive is never stable (if $\alpha = 1$, the nullclines intersect fully and the population may evolve neutrally along a spectrum of strategies).

We may now consider the conditions for which the strategy is an equilibrium along the q_2 axis.

1.2.2 Stability to loss of pure reproduction

Generalist and pure reproductive division of labour is composed of a boundary trait ($q_2 = 0$). As such, the condition to show that the boundary trait is at equilibrium is different. For an interior trait, we need to establish that directional selection of the trait is equal to zero at the putative equilibrium. For a boundary trait, we instead must

show that the directional selection acts to 'push' the trait into the boundary. For our present purposes, this amounts to the condition that $W_{q_2} < 0$ at the putative equilibrium $(p^*, q_1^*, 0)$. Evaluating directional selection in q_2 we find a dependence on α:

$$W_{q_2}(p, q_1^*, 0) = \begin{cases} +\infty, & \text{if } \alpha < 1 \\ (1-p)(-(1-e) - e\lambda p q_1 + e((1-\lambda) + p(1-q_1)\lambda R + \lambda R(1-p))), & \text{if } \alpha = 1 \\ -(1-p)((1-e) + e\lambda p q_1^{\alpha}), & \text{if } \alpha > 1. \end{cases}$$

We thus have that a generalist and pure reproductive division of labour will always be stable to loss of pure reproduction mutations if $\alpha > 1$ and never stable if $\alpha < 1$. Stability if $\alpha = 1$ needs to be evaluated numerically.

1.2.3 Numerical results

We find in our numerical analysis that generalist and pure reproductive is only ever stable to loss of partial division of labour if there are efficiency benefits to specialisation ($\alpha > 1$). We note that stability to loss of pure reproduction is then automatically guaranteed by the above condition for loss of partial division of labour ($\alpha > 1$). We thus have that stability to loss of partial division of labour *guarantees* the stability of generalist and pure reproductive division of labour. As such, this intermediate form of division of labour will be stable so long as uniform cooperation, uniform non-cooperation or sterile helper and pure reproductive division are all unstable.

1.3 Sterile helper and generalist division of labour

Sterile helper and generalist division of labour occurs in the symmetrically equivalent domains $\mathbf{D4} = (0 < p < 1, q_1 = 1, 0 < q_2 < 1)$ and $\mathbf{D5} = (0 < p < 1, 0 < q_1 < 1, q_2 = 1)$. Without loss of generality we only consider the **D4** domain. Just as for the previous strategy, one of the traits here, q_1, is a boundary trait ($q_1 = 1$) and thus is treated

differently. There are again two sources of instability for this strategy: loss of partial division of labour and loss of sterile helping.

1.3.1 Stability to loss of partial division of labour

If we hold $q_1 = 1$ fixed and focus on the interior traits, p and q_2, we may determine whether the strategy is stable to loss of partial division of labour. Analysis proceeds similarly to that for generalist and pure reproductive division of labour. We seek joint values (p^*, q_2^*) in the state space for which directional selection in both traits is zero. We do this by solving the value of q_2 such that the p and q_2 nullclines intersect. In the general model this is done numerically. If we assume that $e = 1$ and $\lambda = 1$, we find the following nullclines:

$$p\text{-nullcline:} \qquad p(q_2) = (q_2^{\alpha}(1+R) - R)/((1-q_2^{\alpha})(1+R))$$

$$q_2\text{-nullcline:} \qquad p(q_2) = q_2^{\alpha-1}(q_2(1+\alpha R) - \alpha R)/((1-q_2^{\alpha-1}(q_2(1+\alpha R) - \alpha R))$$

Solving for an intersection point leads to the following equation: $q_2^{\alpha-1}(\alpha+q_2(1-\alpha))-1 = 0$. If $\alpha = 1$ then the equation is trivially satisfied. In this scenario, the above nullclines intersect fully and thus a spectrum of equilibria exist along which the population may evolve neutrally, including strategies that are not sterile helper and generalist division of labour. We conclude that this strategy is then not an ESS in this case. We also have that $q_2 = 1$ solves the above equation. As this is also not a sterile helper and generalist strategy we may disregard it. If we assume that $\alpha \neq 1$, we then have that the left hand side of the above equation is either monotonically increasing or decreasing function of q_2 depending on the sign of α. In either case, we have that $q_2 = 1$ is the unique solution to the equation and thus that there exists no interior intersection of the nullclines in the simplified model. Thus, if the trait is essential ($e = 1$) and others-only ($\lambda = 1$), we predict that sterile helper and pure reproductive division of labour will never be an ESS.

For the more general model, we determine whether there exists interior equilibria (p^*, q_2^*) numerically.

We now consider whether the equilibrium point is stable to mutations in the boundary trait, q_1.

1.3.2 Stability to loss of sterile helping

Similarly to generalist and pure reproductive division of labour, the strategy is an equilibrium in q_1 as long as $W_{q_1} > 0$ at the putative equilibrium $(p^*, 1, q_2^*)$. Evaluating, we find that $W_{q_1}(p^*, 1, q_2^*) = -p^*((1-e)+e((1-\lambda)+\lambda(p^*+(1-p^*)q_2^{*\alpha})))+(1-p^*)p^*(1-q_2^*)e\lambda\alpha R$, which needs to be solved numerically in order to check that $W_{q_1} > 0$.

1.3.3 Numerical results

We find that sterile helper and generalist is only ever stable to loss of partial division of labour if there is an efficiency benefit to specialisation ($\alpha > 1$). However, we also find that the equilibrium values (p^*, q_2^*) for which this occurs are never stable to loss of sterile helping at the considered parameter values. Therefore, we find no parameter combinations such that sterile helper and generalist is a stable strategy.

1.4 Sterile helper and pure reproductive division of labour

Sterile helper and pure reproductive division of labour strategies occur in the following domains: **D6** $= (0 < p < 1, q_1 = 1, q_2 = 0)$ and **D7** $= (0 < p < 1, q_1 = 0, q_2 = 1)$, where the two domains are symmetrically equivalent. Without loss of generality, we consider only the domain **D6** where phenotype 1 is the sterile helper ($q_1 = 1$) and phenotype 2 is the pure reproductive ($q_2 = 0$). In this scenario, there are now two boundary traits: the levels of cooperation of each phenotype ($q_1 = 1, q_2 = 0$). We therefore treat each trait separately in our analysis. This leads to three sources of instability: loss of cooperation, loss of pure reproduction and loss of sterile helping. These can all be evaluated analytically.

1.4.1 Stability to loss of cooperation

The trait p is the one interior trait of the putative equilibrium and so is treated using the classic, 1 dimensional approach. We seek to determine whether there is a value p^*, for which directional selection in p is zero ($W_p(p^*, 1, 0) = 0$). We note that $W_p(1, 1, 0) < 0$ always and thus if $W_p(0, 1, 0) > 0$, then we may predict that a $0 < p^* < 1$ exists and thus that the putative equilibrium is stable to loss of cooperation ($p^* = 0$) mutations. Evaluated we have that, $W_p(0, 1, 0) = -(1 - e) + e\lambda R$. Solving the inequality gives us:

$$e > 1/(1 + \lambda R).$$

Therefore, a sterile helper and pure reproductive division of labour strategy is always stable to loss of cooperation if the trait is essential ($e = 1$). Otherwise, the strategy is more likely to be stable to loss of cooperation if λ, e and R are high. In this case, solving for $W_p(p^*, 1, 0) = 0$, gives: $p^* = (e\lambda R - (1 - e))/(e\lambda(R + 1)))$. We may then consider whether the strategy is a stable equilibrium in the other two traits.

1.4.2 Stability to loss of sterile helping

Similarly to loss of sterile helping in sterile helper and generalist, a sterile helper and pure reproductive division of labour will be an equilibrium with respect to q_1 if $W_{q_1}(p^*, 1, 0) > 0$. Evaluating gives the condition:

$$W_{q_1}(p^*, 1, 0) = -p^*((1 - e) + e((1 - \lambda) + \lambda p^*)) + (1 - p^*)e\lambda\alpha R p^* > 0.$$

Substituting in the equilibrium value p^* from above then gives the following condition for stability to loss of sterile helping:

$$\alpha > (R + e(1 - \lambda))/(R((1 - e) + e\lambda)).$$

The right hand side of this threshold condition in α is strictly greater than 1 so long as $\lambda \neq 1$. If $\lambda = 1$ then the condition holds that the returns need to be accelerating $\alpha > 1$. Otherwise, we have that stability to loss of sterile helping is more likely as α, R and λ increase and as e decreases.

1.4.3 Stability to loss of pure reproduction

Similarly to loss of pure reproduction in generalist and pure reproductive, a sterile helper and pure reproductive division of labour will be an equilibrium with respect to q_2 if $W_{q_2}(p^*, 1, 0) < 0$. Evaluating gives:

$$W_{q_2}(p^*, 1, 0) = \begin{cases} +\infty, & \text{if } \alpha < 1 \\ (1-p^*)(-(1-e) - e\lambda p^* + e((1-\lambda) + \lambda R(1-p^*))), & \text{if } \alpha = 1 \\ -(1-p^*)((1-e) + e\lambda p^*), & \text{if } \alpha > 1. \end{cases}$$

We see that sterile helper and pure reproductive division is stable to loss of pure reproduction if $\alpha > 1$. If $\alpha < 1$ then the strategy is unstable to loss of pure reproduction. By substituting p^* above, we find that the strategy is not stable to loss of pure reproduction when $\alpha = 1$. We thus find that, if the condition for stability to loss of sterile helping is verified, then the population will be trivially stable to loss of pure reproduction.

1.4.4 Numerical results

We have found analytically that loss of sterile helping is the only form of instability if the social trait is essential ($e = 1$). For non-essential traits ($e \neq 1$), stability to loss of cooperation needs to be satisfied first and then stability to loss of sterile helping. Stability to loss of pure reproduction is never a deciding factor as it is only ever a source of instability if loss of sterile helping is already a factor. For non-essential traits ($e \neq 1$), we find numerically that stability to loss of cooperation is the determining source of instability

at the border of parameter space between the sterile helper and pure reproductive and uniform non-cooperation strategies. In contrast, stability to loss of sterile helping is always the determining source of instability at the border of parameter space between sterile helper and pure reproductive and generalist and pure reproductive (there are no other borders in this case).

1.5 Uniform non-cooperation

The strategy of uniform non-cooperation occurs within the domains: **D8** $= (0 \leq p \leq 1 q_1 = 0, q_2 = 0)$, **D9** $= (p = 0, 0 \leq q_1 \leq 1, q_2 = 0)$ and **D10** $= (p = 1, q_1 = 0, 0 \leq q_2 \leq 1)$, where **D9** and **D10** are symmetrically equivalent. We note that p is a neutral trait in **D8**, q_2 is a neutral trait in **D9** and q_1 is a neutral trait in **D10**. All other traits are boundary traits in the three domains. Since the domains are connected, a population may evolve neutrally along all three domains and thus stability to any mutations in the boundary traits needs to be satisfied for all possible strategies in the super-domain. Without loss of generality we consider only the domains **D8** and **D9**. There are then three sources of instability to uniform non-cooperation: instability to uniform cooperation, instability to rare specialism and instability to weak specialism.

1.6 Stability to invasion by weak specialism

Uniform non-cooperation is stable to weak specialism if the boundary trait q_1 is stable in the domain **D8**. This translates to the condition: $W_{q_1}(p, 0, 0) < 0$ for all values of $p \neq 0$ (at $p = 0$, the population may evolve neutrally onto the **D9** domain). We have that

directional selection in q_1 depends on the value of α:

$$W_{q_1}(p,0,0) = \begin{cases} +\infty, & \text{if } \alpha < 1 \\ p(-(1-e) + e(1-\lambda) + e\lambda R), & \text{if } \alpha = 1 \\ -p(1-e), & \text{if } \alpha > 1. \end{cases}$$

Thus, uniform non-cooperation in this case is trivially stable if $\alpha > 1$ and $e \neq 1$ and is unstable if $\alpha < 1$. If $\alpha = 1$, we have that uniform non-cooperation is stable if $e < 1/(2 + \lambda(R-1))$.

1.6.1 Stability to invasion by uniform cooperation

Uniform non-cooperation is stable to uniform cooperation if the boundary trait q_2, is at equilibrium, uninvadable and convergent stable in the domain **D9**. This translated to the condition $W_{q_2}(0, q_1, 0) < 0$ for all values of q_1. Evaluated, we find a dependence on α:

$$W_{q_2}(0,q_1,0) = \begin{cases} +\infty, & \text{if } \alpha < 1 \\ -(1-e) + e(1-\lambda) + e\lambda R, & \text{if } \alpha = 1 \\ -(1-e), & \text{if } \alpha > 1. \end{cases}$$

Stability along this axis is thus satisfied whenever $\alpha > 1$ and $e \neq 1$. If $\alpha = 1$, then stability holds so long as $e < 1/(2 + \lambda(R-1))$. We thus have, formally, that uniform non-cooperation is never stable if the trait is essential ($e = 1$).

1.6.2 Stability to invasion by to rare specialism

Uniform non-cooperation is stable to rare specialism if the boundary trait p is at equilibrium, uninvadable and convergent stable in the domain **D9**. This translates to the condition: $W_p(0, q_1, 0) < 0$ for all values of $q_1 \neq 0$ (at $q_1 = 0$ the population may evolve

neutrally onto the **D10** axis). Evaluating directional selection in p in this domain gives the following inequality for stability to rare specialism:

$$W_p(0, q_1, 0) = -(1-e) + (1-q_1)((1-e) + e(1-\lambda)q_1^{\alpha}) + e\lambda R q_1^{\alpha} < 0.$$

Directional selection in p equals $-(1-e) + e\lambda R$ if $q_1 = 1$ and thus stability holds at this point if $e < 1/(1+\lambda R)$. Trivially, uniform non-cooperation is always unstable at this point in the simplified model ($e = 1, \lambda = 1$). In the full model, we must then determine if uniform non-cooperation is stable at all internal values of q_1. To do this we solve for all q_1 such that $\partial W_p / \partial q_1(0, q_1, 0) = 0$ and check that $W_p(0, q_1, 0) > 0$ at all these points. This is done numerically.

1.6.3 Numerical results

We find that uniform non-cooperation is unstable to all sources of instability if the cooperative trait is essential ($e = 1$). For non-essential traits ($0 < e < 1$), we find that stability to invasion by rare specialism is always the determining source of instability at the parameter space border with all other strategies.

1.7 Uniform cooperation

Uniform cooperation occurs in the following connected domains: **D11** $= (p = 1, 0 < q_1 < 1, 0 \leq q_2 \leq 1)$, **D12** $= (p = 0, 0 \leq q_1 \leq 1, 0 < q_2 < 1)$ and **D13** $= (0 \leq p \leq 1, 0 < q_1 < 1, q_2 = q_1)$, where **D11** and **D12** are symmetrically equivalent domains. We have that q_2 is a neutral trait in **D11**, q_1 is a neutral trait in **D12** and p is a neutral trait in **D13**. As such, the population may evolve neutrally along all three domains at a fixed cooperative investment, q^*, and stability thus needs to be shown for all points along the super-domain. There are then three sources of instability that need be considered, loss of cooperation (a stable cooperative investment, q^*, does not exists), instability to rare specialism and

instability to weak specialism.

1.7.1 Stability to loss of cooperation

Uniform cooperation is stable to loss of cooperation if there exists a non-zero cooperative investment, q^*, for a population of monomorphic generalist helpers that is both an equilibrium, uninvadable and convergent stable. This may be calculated as the value q^* for which $W_{q_1}(1, q_1 = q^*, 0 \leq q_2 \leq 1) = -((1-e)+eq^{*\alpha})+(1-q^*)e(1-\lambda)\alpha q^{*\alpha-1}+(1-q^*)e\lambda R\alpha q^{*\alpha-1} = 0$. If this q^* is non zero (and less than one) then the strategy is stable to loss of cooperation. This needs to be calculated numerically in the full model. If we make the simplifying assumptions that $e = 1$ and $\lambda = 1$ then we find the following analytical result: $q^* = \alpha R/(1 + \alpha R)$, which is strictly less than 1, greater than 0 as long as $a < 1$.

1.7.2 Stability to invasion by rare specialism

Uniform cooperation is stable to rare specialism if the boundary trait, p, is an equilibrium in the domain **D11** ($p = 1$), for all possible values of $q_2 \neq q_1$ and where $q_1 = q^*$ is the equilibrium cooperative investment calculated numerically in the full model. At the point $q_2 = q_1$, the population is allowed to evolve neutrally onto the **D13** domain. This translates to the condition $W_p(1, q^*, q_2) > 0$ for all $q_2 \neq q^*$. Evaluating we find: $W_p(1, q^*, q_2) = (1-q^*)((1-e)+eq^{*^\alpha})-(1-q_2)((1-e)+e((1-\lambda)q_2^\alpha+\lambda q^{*^\alpha}))+(1-q^*)e\lambda R(q^{*^\alpha}-q_2^\alpha)$ We check numerically that $W_p(1, q^*, q_2) > 0$ at the boundary values $q_2 = 0$ and $q_2 = 1$ as well as at any internal points, $0 < q_2 < 1$ that may minimise $W_p(1, q^*, q_2)$ (solve for q_2 values such that $\partial W_p/\partial q_2(1, q^*, q_2) = 0$). For example, the following is the stability condition at the boundary $q_2 = 0$:

$$W_p(1, q^*, 0) = (1-q^*)((1-e)+eq^{*^\alpha})-((1-e)+e\lambda q^{*^\alpha})+(1-q^*)e\lambda R q^{*^\alpha} > 0$$

In the simplified model ($e = 1, \lambda = 1$), we find that the unique value of q_2 that solves

$\partial W_p/\partial q_2(1, q^*, q_2) = 0$ is $q_2 = q^*$ and that $W_p(1, q^*, q^*) = 0$. This is simply the neutral instability that is allowed by the construction the model. In order to determine whether the population is unstable at other values of q_2 (including the boundaries) we need simply determine whether $q_2 = q^*$ represents a minimum or maximum of directional selection in p. If it is a minimum, then all other values of q_2 satisfy $W_p(1, q, q2) > 0$ and thus the strategy is stable and, if it is a maximum, then all other values of q_2 lead to negative directional selection in p and thus the population is unstable to weak specialism. We determine this by evaluating the sign of $\partial^2 W_p/\partial q_2^2(1, q^*, q^*)$ and find that uniform cooperation is stable in the case that $\alpha < 1$ and unstable if $\alpha \geq 1$ (for the simplified model).

1.7.3 Stability to invasion by weak specialism

Stability to weak specialism occurs if the interior traits $q_1 = q^*$ and $q_2 = q^*$ are jointly an equilibrium in the domain **D12** for all possible values of $0 < p < 1$. If we can show that $W_{q_1}(p, q^*, q^*) = 0$ for all values of p then both q_1 and q_2 are an equilibrium (by symmetry of the levels of cooperation of each phenotype). If we assume that the trait is essential ($e = 1$) and others-only ($\lambda = 1$), then we find that $W_{q_1}(p, q^*, q^*) = 0$ for all p.

1.7.4 Numerical results

At the parameter space border with other possible strategies, we find that the determining source of instability for whether uniform cooperation is favoured is always instability to invasion by rare specialism. In particular, uniform cooperation is stable so long as it is uninvadable by a rare mutant strain with a division of labour between a helper reproductive ($q_1 = q^*$) and a pure reproductive ($q_2 = 0$). We found previously that uniform cooperation is the only non-division of labour strategy that may be stable if the cooperative trait is essential ($e = 1$). Thus, if uniform cooperation is unstable in this regime ($e = 1$), then a division of labour strategy is the equilibrium strategy (by exclusion.) If the trait is non-essential ($e < 1$) and uniform cooperation is not stable, then

either a division of labour strategy or uniform non-cooperation are the equilibrium.

2 Supplementary information: uninvadability of the equilibria

In order for an equilibrium strategy to be an ESS, we must show that a population otherwise at equilibrium is uninvadable by a rare mutant lineage with an alternate strategy. It is sometimes claimed that the uninvadability of an equilibrium point cannot be mathematically demonstrated in kin selection models without specifying the genetic details of the system or without recourse to a dynamical model or computer simulation[8,28]. Here we used an ESS condition derived elsewhere (Cooper and West, in preparation) that does match the ESS condition derived using the simplest genetic model (asexual, haploid) for patch-structured populations as found in Taylor and Day[8].

We begin by evaluating the analytical uninvadability conditions for each trait in our model and show that all of our equilibria satisfy these conditions for each trait that may vary. However, we also verify computationally that our division of labour equilibria are uninvadable. First, we check numerically for each of the division of labour equilibria that mutant lineages with respect to each trait would indeed have lower fitness than that of an arbitrary individual in the equilibrium population. Second, we employ individual based simulations to show that a sampling of our division of labour equilibria are indeed stable points of the dynamical system. We note that this entire analysis hinges on the assumptions that (a) relatedness is an independent parameter of the model and (b) competition for niches is global.

2.1 Analytical conditions

The condition for uninvadability is that is is a local maximum of the fitness landscape[25,43]. Let x be the genic value of a rare mutant. In a population with equilibrium genic value x^*,

we then have that the uninvadability condition amounts to evaluating the second order of fitness with respect to the mutant genic value[8,14,28,43]:

$$\frac{d^2W}{dx^2}\Big|_{x=x^*} < 0.$$

If this second derivative is negative, then we have that the fitness landscape is concave around the equilibrium and therefore that rare mutants have lower fitness than individuals at equilibrium.

We derive uninvadability by a simple extention of the Taylor-Frank approach[8,45]. Assume that the population is infinite and structured into finite patches of uniform size. Let the genic value x determine the phenotype of the mutant, y, and influence the average phenotype of the focal mutant's patch, Y, (via the phenotype of all patch-mates that are IBD to the focal mutant.) Let y^* then be the equilibrium phenotype that corresponds to x^*. In this case, the fitness of a rare mutant in a patch may be expressed as $W(x) = W(y, Y)$ and we may evaluate the second-order condition above by applying the chain rule. This gives the following uninvadability condition for a population at the equilibrium y^*:

$$\Big(\frac{\partial^2 W}{\partial y^2} + 2\frac{\partial^2 W}{\partial y \partial Y}R + \frac{\partial^2 W}{\partial Y^2}R^2\Big)\Big|_{y=Y=y^*} < 0,$$

where $R = \frac{dY}{dx}/\frac{dy}{dx}$ and higher order derivatives of genic value on phenotype are assumed to be negligible (Cooper and West, in preparation)[8,14,25,28,43,45]. This assumption is permissible because we are only considering global competition and do not consider the effect of trait mutations on relatedness in the population (R is fixed and independent).

Below, we evaluate this ESS condition for each of the traits of our model:

Uninvadability in p:
$$2R(q_2 - q_1)e\lambda(q_1^\alpha - q_2^\alpha) < 0$$

Uninvadability in q_1:
$$(1-\lambda)((1-q_1)(\alpha-1) - 2q_1) - 2Rp\lambda q_1 + R^2\lambda(\alpha-1)(p(1-q_1) + (1-p)(1-q_2)) < 0$$

Uninvadability in q_2:
$$(1-\lambda)((1-q_2)(\alpha-1) - 2q_2) - 2R(1-p)\lambda q_2 + R^2\lambda(\alpha-1)(p(1-q_1) + (1-p)(1-q_2)) < 0$$

We affirm that these conditions should only be applied to interior traits of the equilibrium. If a trait of an equilibrium lies at the boundary, then the equilibrium is negligibly uninvadable with respect to that trait due simply to the equilibrium condition that directional selection in that trait is directed into the boundary.

If a trait of an equilibrium is neutral, such that the population may evolve neutrally along that trait axis without altering the underlying strategy, the uninvadability with respect to that trait need not be shown.

Across all of the equilibria that we identify in our model, we found using the above conditions that each equilibria is also uninvadable and thus is an ESS of the system.

2.2 Numerical verification

In our analysis, we discretised parameter space into $205,209$ nodes. Amongst these, we identified $54,611$ division of labour equilibria ($35,276$ sterile helper and pure reproductive equilibria and $19,335$ generalist and pure reproductive equilibria.) We found that each of these division of labour equilibria satisfied our analytical conditions for uninvadability. However, we can also numerically verify that these equilibria are maxima of the fitness

landscape (and not minima.)

We do this by considering each equilibrium in turn, (p^*, q_1^*, q_2^*) and its associated parameter values (α, λ, R, e), and evaluating the relative fitness of a series of mutant lineages that each deviate a given amount from only one of the traits at a time. In our analysis, we considered 20 mutant lineages for each trait that varied ($\pm\epsilon$, $\pm 2\epsilon$, ..., $\pm 10\epsilon$) from the equilibrium value of the trait where $\epsilon = 0.01$. However, we did not consider mutant lineages if the perturbed trait value falls outside of the boundaries of our model.

For a given equilibrium, if the relative fitness of all considered mutant lineages was less than the fitness of a neutral mutant (with no change in trait values) then we may conclude that the equilibrium strategy is a maximum of the local fitness landscape and is therefore uninvadable. In Supplementary figure 1, we illustrate this analysis for two such equilibria, a generalist and pure reproductive equilibrium and and sterile helper and pure reproductive equilibrium. We see that in both cases, all mutant lineages have lower fitness than the equilibrium strategy, which we thus deem uninvadable in both cases.

We repeated this analysis for all $54,611$ division of labour equilibria and found in each case that the equlibrium (neutral) strategy always had a higher fitness than any of the mutant lineages and thus affirm that they are all ESSs of the fitness landscape.

2.3 Dynamic individual-based simulations

We now show that a sampling of the division of labour equilibria are stable using dynamic individual-based simulations. For each equilibrium considered, we check whether a monomorphic population at that equilibrium is uninvadable by a rare mutant deviating from the equilibrium in a single trait value by an amount $\epsilon = 0.05$. We do this for each possible trait value that may vary, repeating the invasion simulation a total of $L = 10,000$ times for each possible mutant lineage. We then record the fraction of simulations for which the particular mutation either went extinct or evolved to fixation (or neither outcome) by the end of $T = 10,000$ generations.

We employ a haystack model for our simulations wherein we presume that relatedness is fixed. This simplifies the computation and means that we do not need to explicitly model the size that groups grow to after founding. As such, we need only model the founding individuals of each group.

Consider a population with $M = 10,000$ groups of $F = 1/R$ founding individuals. Let all founding individuals have the equilibrium strategy (p^*, q_1^*, q_2^*), save one founding individual in an arbitrary group with mutant strategy (p', q_1', q_2').

For T timesteps, repeat the following:

1. Calculate the average p, q_1 and q_2 in each group.
2. Calculate the expected fitness, W, of each individual in the population.
3. Draw $F \times M$ random individuals (with replacement) from the population with probabilities equal to their fitness, W.
4. Assort these individuals randomly into M groups of F individuals. These are then the founding individuals of the next generation.

At each step of the above iteration, we keep track of the number of mutant individuals in the population. If at some point, the number of mutant individuals falls to 0, then it will stay at 0 for the rest of the simulation and we say that the mutant lineage has gone extinct. On the other hand, if the number of mutants rises to $M \times F$ individuals, then it will stay at this number and we say that the mutation has evolved to fixation.

In Supplementary figure 2, we use simulation to show the uninvadability of a sterile helper and pure reproductive division of labour equilibrium to a mutant lineage with a perturbed helper probability. We see that over $L = 10,000$ simulated invasions, all mutant lineages have gone extinct by the end of $10,000$ generations.

We performed the above analysis for each of our 60 division of labour equilibria (42 sterile helper and pure reproductive equilibria and 18 generalist and pure reproductive

equilibria), considering fixed mutations along each of the permissible trait axes. The results of this analysis are shown in Supplementary figure 3. We found that all $10,000 \times 4 \times 42$ simulations for the sterile helper and pure reproductive equilibria had mutant lineages that all went extinct. We also found that no single mutant invaded to fixation in the generalist and pure reproductive equilibria ($10,000 \times 5 \times 18$ simulations). However, 6 of the generalist and pure reproductive equilibria did have $1-2$ mutant lineages (out of $10,000$) that were not extinct by the end of the simulation. Strictly speaking, this does not meant that these equlibria are invadable by mutations but rather that they are weakly uninvadable (shallow maxima of the fitness landscape.) Indeed, we found that on average even neutral mutations would have about 2 mutant lineages invade to fixation out of $10,000$ simulations simply due to stochastic drift. In Supplementary figure 3, we see that all of these weakly uninvadable division of labour equilibria are near the parameter space boundary with non-division of labour strategies which is to be expected.

3 Supplementary information: convergence stability

An equilibrium strategy is *convergent stable* if it is an attractor of the dynamical system: a monomorphic population close to the equilibrium strategy always experiences directional selection that favours a rare mutant lineage with trait value closer to the equilibrium[9]. For each parameter combination considered in our numerical discretization, we also checked that the associated equilibrium is convergent stable. We use the methodology developed by Brown and Taylor (2010). That is, the first derivative of directional selection in each interior trait (non-boundary traits) that is non neutral must be negative and the jacobian of directional selection of all possible combinations of interior traits must be negative definite. The convergence stability of neutral traits need not be considered as the population is free to evolve along these axes without altering the underlying strategy. Boundary traits of an equilibrium, meanwhile are negligibly convergent stable due to the fact that directional

selection in that trait is directed into the boundary (by the equilibrium condition.)

The strategy comprising two different generalist types is the only strategy for which all traits are interior traits and non-neutral. However, no equilibrium with respect to this strategy was identified in our analysis and so the conditions for convergence stability in this case are never applied. All other strategies contain at least one boundary or one neutral trait. For example, the strategy of generalist and pure reproductive consists of one boundary trait ($q_2 = 0$ without loss of generality) and two non-neutral, interior traits (p and q_1). As such, the first derivative of directional selection in p and q_1 (with respect to that trait) must be shown to be negative. In addition, the jacobian of directional selection in both traits must be shown to be negative definite, which amounts to showing that the determinant of the jacobian is negative[5]. The strategy of uniform non-cooperation consists of two boundary traits and one neutral trait and thus is negligibly convergent stable if the equilibrium condition is satisfied. The strategy of uniform cooperation is treated differently depending on which of the domains is considered. In the domain, **D11**, there is one boundary trait ($p = 1$), one neutral trait (q_2) and one interior trait that must be analysed singly (q_1). However, in the domain **D13**, p is a neutral trait whereas q_1 and q_2 are interior traits. As such the the convergence stability of both interior traits must be shown for all values of p. This is done numerically for a fine discretization of p for each uniform cooperation equilibrium.

Across all discretized parameter combinations of analysis, all of the associated equilibria were found to be convergent stable.

4 Supplementary information: non-linear costs to cooperation

Our model presumes that the costs of cooperative investment are linear; we consider only nonlinear effects upon the beneficial returns from cooperation (α). Here we present

a generalized fitness function that includes a non-linear cost to cooperation. We show that a simple change of variables can yield the same qualitative fitness function as was considered previously. Let the generalized fitness function be:

$$W = p(1-q_1^\beta)\Big[(1-e)+e\big((1-\lambda)q_1^\alpha+\lambda(PQ_1^\alpha+(1-P)Q_2^\alpha)\big)\Big] \\ +(1-p)(1-q_2^\beta)\Big[(1-e)+e\big((1-\lambda)q_2^\alpha+\lambda(PQ_1^\alpha+(1-P)Q_2^\alpha)\big)\Big],$$

where the parameter $\beta > 0$ is the shape of the costs of cooperation. We now propose the change of variables: $k_1 = q_1^\beta$ and $k_2 = q_2^\beta$. Thus we are now considering the cost off cooperation as an explicit variable in our model rather than the underlying cooperative investment. The variables Q_1 and Q_2 are the others-only averages of cooperative investment for each phenotype in the focal social group. If we consider that selection is weak, then the variance in cooperative investment across all individuals of the same phenotype in the group will be nearly negligible. As such, Q_1 and Q_2 may be calculated using the geometric mean rather than the arithmetic mean. Let m_1 and m_2 be the others-only number of individuals in the social group of phenotypes 1 and 2 respectively. Assume that individuals of each phenotype may be indexed by i. For example, q_{1i} (k_{1i}) is the cooperative investment (cost of cooperation) of the ith individual of phenotype 1. This gives the following for phenotype 1:

$$Q_1 = \Big(\prod_{i=1}^{m_1} q_{1i}\Big)^{1/m_1} = \Big(\prod_{i=1}^{m_1} k_{1i}\Big)^{1/m_1\times 1/\beta} = K_1^{1/\beta},$$

where K_1 is the others-only average cooperative cost payed by individuals of phenotype 1 in the social group. The same logic can be used to show that $Q_2 = K_1^{1/\beta}$. If we then

substitute these back into the fitness equation we arrive at:

$$W = p(1-k_1)\Big[(1-e)+e\big((1-\lambda)q_1^{\alpha/\beta}+\lambda(PK_1^{\alpha/\beta}+(1-P)K_2^{\alpha/\beta})\big)\Big] + (1-p)(1-k_2)\Big[(1-e)+e\big((1-\lambda)q_2^{\alpha/\beta}+\lambda(PK_1^{\alpha/\beta}+(1-P)K_2^{\alpha/\beta})\big)\Big],$$

which has the same functional form as the original fitness function and thus yields the same qualitative results. In this generalized fitness function, α, is replaced by α/β which is the ratio of the shape of the returns from cooperation to that of the costs. As such, the condition $\alpha > 1$ no longer signifies that the returns must be accelerating but translates to: the returns from cooperation must accelerate more (or diminish less) than the costs of cooperation.

5 Supplementary information: the evolution of extreme specialisation

We hypothesise that there may be an evolutionary feedback loop in which helper specialisation drives reproductives to help less and reproductive specialisation drives helpers to help more. In particular, if we consider the effect of a change in the cooperative investment of one phenotype upon directional selection in the cooperative investment of the other phenotype we find that:

$$\frac{\partial W_{q_1}}{\partial q_2} = -p\lambda(1-p)\alpha q_2^{\alpha-1} - (1-p)e\lambda p\alpha q_1^{\alpha-1}R,$$

which is always negative. Similarly, we can show that $\frac{\partial W_{q_2}}{\partial q_1} < 0$. As such, a monomorphic decrease in the cooperative investment of the less cooperative phenotype leads to selective pressure for an increase in the cooperative investment of the more cooperative phenotype and vice versa. However, this is not dynamically sufficient analysis and so is not conclusive.

In order to test this hypothesis, we performed a series of dynamic, individual based simulations. Our simulations were similar in form to those performed in the uninvadability analysis except that random mutations in the trait value of some of the traits (evolving traits) may accrue. The non-evolving traits are fixed at their starting values and dot not accrue mutations. Consider a population with M groups of $F = 1/R$ founding individuals. Let all individuals in the initial population have the starting strategy (p, q_1, q_2). Broadly, a single simulation is composed of the following iteration.

For T timesteps, repeat the following:

1. Calculate the average p, q_1 and q_2 in each group.

2. Calculate the expected fitness, W, of each individual in the population.

3. Draw $F \times M$ random individuals (with replacement) from the population with probabilities equal to their fitness, W.

4. With probability μ each individual may experience a mutation in one of the evolving traits (p, q_1 or q_2) where the mutation is randomly drawn from a normal distribution with variance σ.

5. Assort these individuals randomly into M groups of F individuals. These are then the founding individuals of the next generation.

With this setup we performed the following analyses. In Figure 4a, we fixed reproductive cooperation (q_2) at high and low values and allowed the level of cooperation of the helper (q_1) to evolve from a starting value of the ancestral expected value of cooperation $(R\lambda + (1-\lambda))/(1 + \alpha(R\lambda + (1-\lambda))$. In Figure 4b, we fixed helper cooperation (q_1) at high and low values and allowed the level of cooperation of the reproductive (q_2) to evolve from a starting value of the ancestral expected value of cooperation. In Figure 4d, we fixed reproductive cooperation to $q_2 = 0.25$ and allowed only helper cooperation (q_1) to evolve

for $10,000$ generations after which the levels of both helper and reproductive cooperation (q_1 and q_2) were allowed to evolve for another $10,000$ generations. In Figures 4a, 4b & 4d, we repeated each simulation a total of 25 times, averaging results across trials and present 95 percent confidence intervals based on normal distribution. In Figure 4c, we fixed the level of cooperation of one phenotype over a series of values, allowed the level of cooperation of the other phenotype to evolve and recored the population average level of cooperation of the evolving phenotype at the end of 3000 generations. We repeated this simulation for each fixed level of cooperation at total of 10 times, averaged results across trials and presented 95 percent confidence intervals. In Figures 4a-d, we set the number of groups to $M = 5000$, we set the mutation rate to $\mu = 0.01$ per evolving trait per generation, we set the variance of mutation size to $\sigma = 0.01$ and we fixed the probability of being one phenotype over the other to $p = 0.5$. The same analysis we performed with an evolving phenotype probability (p) and the same qualitative results were found (both phenotypes always evolve to full specialisation but the speed at which they do so depends on the level of cooperation of the other phenotype; the phenotype probability evolves to a value that reflects the fixed level of cooperation). In all figures, we set $\alpha = 2$, $e = 1$ and $\lambda = 1$. For Figures 4a and 4b we set $R = 1/3$ and for Figures 4c and 4d we set $R = 1/2$. The parameter values of each analysis were chosen such that sterile helper-pure reproductive division of labour was the favoured strategy and for ease of interpretation.

6 Supplementary information: a closed model of division of labour

In our analysis, we have employed an open model approach wherein the demographic processes that generate relatedness have not been specified. Instead, we leave R as an independent parameter of the model and show how the evolution of division of labour depends on the value of R. The benefit of this approach is that it may lead to general

predictions that hold regardless of the specific way that relatedness may arise within populations. However, an open model may fail to make accurate predictions for specific systems in which there are significant interactions between R and other factors modelled. This shortcoming is overcome by a closed model approach in which demographic detail is specified and and relatedness is solved for as a function of these processes. This allows for a more detailed model that can capture interactions between relatedness and other feature of the model. However, the downside of a closed model approach is that any predictions made will then only be applicable to biological systems that match the demographic assumptions of the closed model. As such, the trade-off between using an open or closed model approach is that of demographic precision against broad applicability.

The purpose of our work was to explain the evolution and diversity of division of labour across the tree of life, regardless of the specific mechanisms by which relatedness arises. As such, an open model was the more appropriate approach for our purposes. To illustrate the conclusions that may be drawn from a closed model, we now analyse a simplification of our division of labour model using a closed approach.

6.1 Extending the open model

For the purposes of analytical tractability, we first assume that al helpers are sterile (q_1=1) and that all reproductives are pure reproductives (q_2=0). As such we seek candidate ESS values of p, the probability of becoming a sterile helper. For a particular combination of model parameters, if we find that $p^* > 0$ then we conclude that division of labour between a sterile helper and pure reproductive is the ESS strategy and otherwise we conclude that uniform non-cooperation is the ESS. In the open model analysis, we found that sterile helper and pure reproductive division of labour was stable to invasion by uniform non-cooperation if a threshold condition in trait essentiality was met ($e > 1/(1 + \lambda R)$; Supplementary figure 4 depicts this threshold condition for $\lambda = 1$.)

Now suppose that we wanted to model the demographic processes that generate re-

latedness between interaction individuals. After each generation, if offspring individuals have a probability s of staying (and competing for niches) on their natal patch (social group) and a probability $1 - s$ of dispersing to a different patch in the population (and competing for niches there), then this limited dispersal will lead to a buildup of of genetic correlations over time amongst the individuals that stay on their natal patches. An additional way to generate relatedness between individuals is to have overlapping generations. If individuals have a probability k of surviving (and retaining their niches) from one generation to the next or of reproducing and dying (with probability $1 - k$), then this means that IBD individuals from different generations may interact which represents an increase in social group relatedness. In combining these two processes (limited dispersal and non-overlapping generations), we have from Taylor and Irwin that the long-term whole-group relatedness is given by:

$$R_{wg} = \frac{1 + k}{N + kn + 2ks - 2kns + s^2 - ks^2 - ns^2 + kns^2},$$

where N is the number of niches on a patch[46]. This may be used to calculate the others-only relatedness by the transformation: $R = (R_{wg} - 1)/(N - 1)$. Broadly this formulation means that relatedness increases the more that individuals stay on their natal patches (high s), the more that generations overlap (high k) and for smaller social groups (low N). These effects are depicted in Supplementary figure 5.

6.2 Accounting for the demographic processes

We may now begin to reformulate our fitness equation for the closed model. We first have that the payoff to an individual with trait value p on a patch with (others-only) average

trait value P is given by

$$G(p,P) = (1-p)\Big((1-e) + e\lambda P\Big)$$

We then have that the fitness of an individual will depend upon whether it survives from one generation to the next and on the competitive environment that offspring face (depending on whether they stay or disperse from the natal patch.) If we assume that p^* is the population wide average trait value and that ρ is the (group-wide) average trait value in the focal patch ($\rho = (p + (N-1)P)/N$) then the fitness of a focal individual is:

$$W(p,P) = k + (1-k)s\frac{G(p,P)}{sG(\rho,\rho) + (1-s)G(p^*,p^*)} + (1-k)(1-s)\frac{G(p,P)}{G(p^*,p^*)}$$

where the first term is the probability that the focal individual survives. Otherwise, the second term is the fitness due to offspring that compete on the natal patch and the third term is the fitness due to offspring that disperse and compete for niches on other patches in the population. We solve for a candidate ESS using the Taylor-Frank methodology[45]:

$$\begin{aligned}\Big(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\Big)\Big|_{p=P=p^*} = \quad & \frac{1-k}{G(p^*,p^*)}\Big((1-s^2/N)G_1(p^*,p^*) - (s^2/N)G_2(p^*,p^*))\Big) \\ + \quad & R\frac{1-k}{G(p^*,p^*)}\Big((1-((N-1)/N)s^2)G_2(p^*,p^*) \\ - \quad & ((N-1)/N)s^2G_1(p^*,p^*))\Big) \\ = 0 \quad & ,\end{aligned}$$

where $G_1(p,P) = -((1-e) + e\lambda P)$ and $G_2(p,P) = (1-p)e\lambda$ are the first derivatives of $G(p,P)$ with respect to the first and second variables (p and P), respectively. We note that the above equation is still an open model. While further demographic detail has been included in the fitness equation, R is still present as an independent parameter in the model. In order to fully 'close' the model, we must substitute in R as a function of s,

k and N as calculated earlier before solving for the equilibrium value of p^*.

6.3 Closed model results

Setting $\lambda = 1$, we numerically discretised the parameters e, s, k and N and solved for each combination of parameter values whether the equilibrium valued of $p^* > 0$. The results of this analysis are plotted in Supplementary figure 6, where we see that a similar threshold condition in e is recovered as was found for the open model (Supplementary figure 4.) We find that division of labour between a sterile helper and pure reproductive is favoured over uniform non-cooperation if the demographic parameters s and k are high and if N is low. Incidentally, limited dispersal (high s), overlapping generations (high k) and small group sizes (low N) are exactly the demographic conditions that lead to high social group relatedness and thus we have qualitatively recovered the same interaction between trait essentiality and social group relatedness as found in the open model (supplementary figure 4.) Additionally, we find that when there are non-overlapping generations ($k = 0$), division of labour is never favoured regardless of the values of s or N (not depicted). This recovers the well-known result that limited dispersal alone cannot favour cooperation in this way as the indirect fitness benefits due to an increase in relatedness is exactly cancelled by the competitive costs of related offspring competing for niches on the natal patch[44]. The inclusion of overlapping-generations has previously been shown to remove this effect[23,46].

7 Supplementary information: further predictions of the model

7.1 The efficiency benefits of specialisation.

We found in our model that an efficiency benefit to specialisation ($\alpha > 1$) is necessary, but not sufficient for division of labour (Figure 3)[13,52]. This occurs because other factors

may hinder the favourability of division of labour. For example, if there is limited sharing of the benefits of cooperation (lower λ), then it may not pay to divide labour, despite high benefits of specialisation ($\alpha > 1$).

We find that, in most scenarios, division of labour and sterile helping are favoured by an increase in the efficiency benefits of specialisation (higher α; Figure 3). However, if the interplay with other factors (lower e, λ and R) means that the benefits of cooperation are relatively minor, then an increase in the efficiency benefits of specialisation (higher α) may actually disfavour division of labour. This prediction, in the opposite direction to that usually found, arises because uniform non-cooperation may be favoured instead (Figure 3). Indeed, a greater efficiency benefit to specialisation (higher $\alpha > 1$) means that it can pay to defect as significant benefits to cooperation can only be obtained at a high private cost (higher q, Figure 2b).

Empirically, there have been no formal tests of the influence of the efficiency benefit to specialisation on whether division of labour is favoured and the form it takes. Our predictions suggest that not only is such a test required, but that it would be useful to look at how the efficiency benefits (α) interplay with social group relatedness (R), the essentiality of the trait (e) and the trait sociality (λ).

7.2 How are the benefits of cooperation shared?

In all cases, we find that an increase in trait sociality (higher λ) favours division of labour and helper sterility (Figure 3). At higher trait sociality (higher λ), less of the benefits of cooperation are directed to those who cooperate, making it more efficient to divide between helpers and reproductives and less costly for helpers to become sterile.

Although this predicted role of sociality has not been directly tested, it is consistent with the pattern across microbes. In microbes, sociality is likely to vary in a predictable way across different types of traits. In species that form fruiting bodies to aid dispersal, such as slime moulds, the 'lifting up' of other cells will benefit other cells, and not the

'stalk' cells that do the lifting, leading to $\lambda \approx 1$ (others-only trait)[41]. In contrast, when bacterial cells produce and release public goods, the benefits are likely to be either shared equally amongst the local group (whole-group trait), or may go preferentially to the cell that produced them[16,22,48,51]. This would lead to $\lambda \leq (N-1)/N$, where N is the number of cells in the social group. Consequently, we predict that division of labour is less likely with public goods. Consistent with this prediction, bacteria produce many public goods without division of labour, and the example of intermediate division between a generalist and pure reproductive in *B. subtilis* is over a public good[48,51].

7.3 How essential is cooperation?

Many previous models of division of labour have assumed that cooperation is essential for survival and reproduction ($e = 1$; Supplementary table 1). However, this will often not be the case. While individuals in groups that lack cooperators may do less well, they do not necessarily have a fitness of zero[11,16,19,32,37,38,40,41]. The extent to which a trait is essential will vary depending on the environment of a system. Cooperative traits relating to resource acquisition or defense may be more or less essential depending on resource availability or the threat of predation in the local environment. In our model, we find that the essentiality of the trait (e) has multiple influences, such that a higher essentiality can either favour or disfavour division of labour (Figure 3).

On the one hand, in systems where cooperation is largely favoured (higher R and λ) a decrease in trait essentiality (lower e) favours division of labour and helper sterility (Figure 3a-3c). Division of labour is favoured in this scenario because it is then less costly to have pure reproductives that do not invest in cooperation. Helper sterility is favoured by a decrease in trait essentiality (lower e) because this leads to a lower proportion of helpers (lower p^*) which triggers higher levels of helper cooperation in compensation (higher q_1^*).

On the other hand, as the trait becomes less essential (lower e), it also makes it

easier for the strategy of uniform non-cooperation to outcompete cooperation (Figure 3c and 3d). Consequently, with less essential traits (lower e), division of labour may be more favourable than uniform cooperation but uniform non-cooperation may become more stable than either strategy, particularly for low relatedness (Figure 3d). Overall, we find numerically that the more dominant effect of a lower trait essentiality (lower e) is to disfavour division of labour and helper sterility (Figure 3d).

However, these opposing effects makes it difficult to make broad predictions for what should be observed empirically. This problem can be overcome by focusing on biological systems in which uniform non-cooperation is never observed. In this case, we can make the clear prediction that both division of labour and a sterile helper are more likely with less essential traits (lower e). The effect of the essentiality of the cooperative trait on division of labour has not been tested empirically in either animals or microbes.

7.4 What is the optimal proportion of helpers?

Across biological systems that employ division of labour, there is notable variation in the ratio of helpers to reproductives (p^*) and our model can predict the factors that contribute to this variation (Figure 5). In some cases, the predicted trend is consistent. A higher social group relatedness (higher R) and higher trait essentiality (higher e) both lead to a division of labour with a higher proportion of helpers (higher p^*). This occurs because higher relatedness and trait essentiality both increase the indirect benefits from helping relatives in the social group.

Consistent with our predicted influence of relatedness, species in which groups of cells are formed clonally ($R = 1$) have approximately three times the proportion of sterile helpers as species that form non-clonal groups ($R < 1$)[10]. However, this difference is based on a small number of phylogenetically independent comparisons, and hence lacks statistical power. Further data is required from phylogenetically diverse groups.

In contrast, the direction of the predicted relationship with other factors can depend

upon the form of division of labour that is favoured. Considering the influence of trait sociality (λ), we find that a higher sociality (higher λ) leads to more helpers (higher p^*) when the helpers are generalists ($0 < q_1^* < 1$), but fewer helpers (lower p^*) when the helpers are fully specialised and sterile ($q_1^* = 1$; Figure 5b). Analogously, considering the influence of the efficiency benefit from greater cooperation (α), we find that a higher efficiency benefit (higher α) leads to fewer helpers (lower p^*) when the helpers are generalists ($0 < q_1^* < 1$) but has no influence on the fraction of helpers when the helpers are sterile ($q_1^* = 1$; Figure 5a). These different predictions arise because, when there are generalists, the amount that they help (q_1^*) also changes (Figures 5c and 5d). So for example, with a high efficiency benefit (higher α), we predict few generalists (lower p^*) but who help a lot (high q_1; Figure 5c).

These predictions about the relative investment into helpers and reproductives have not been tested. In microbes, the proportion of the different phenotypes varies widely, both between and within species, allowing numerous opportunities for such tests[4,20,21,49,50]. In the social insects, there is a rich theoretical and empirical literature examining the ratio of workers belonging to different behavioural castes[15,31,53,55]. The optimal ratio of different castes of helpers is a different problem from our focus on the division between helpers and reproductives. Nonetheless, a general pattern from the social insect work is that the proportion of a workers in a caste decreases as the caste becomes more specialised and thus fewer workers are needed to perform the associated tasks[31,55]. This is similar to our result that an increase in the benefits of specialisation or the sociality of the trait (higher α or λ) lead to both an increase in specialisation (higher q_1^*) and a decrease in representation in the group (lower p^*). In contrast, we found that when an increasing relatedness (R) drives increasing helper investment (higher q_1^*), then the proportion of helpers actually increases as well (higher p^*). This difference arises because increased efficiency benefits and trait sociality (higher α and λ) favours more specialised division of labour whereas increased relatedness (higher R) favours more cooperation overall.

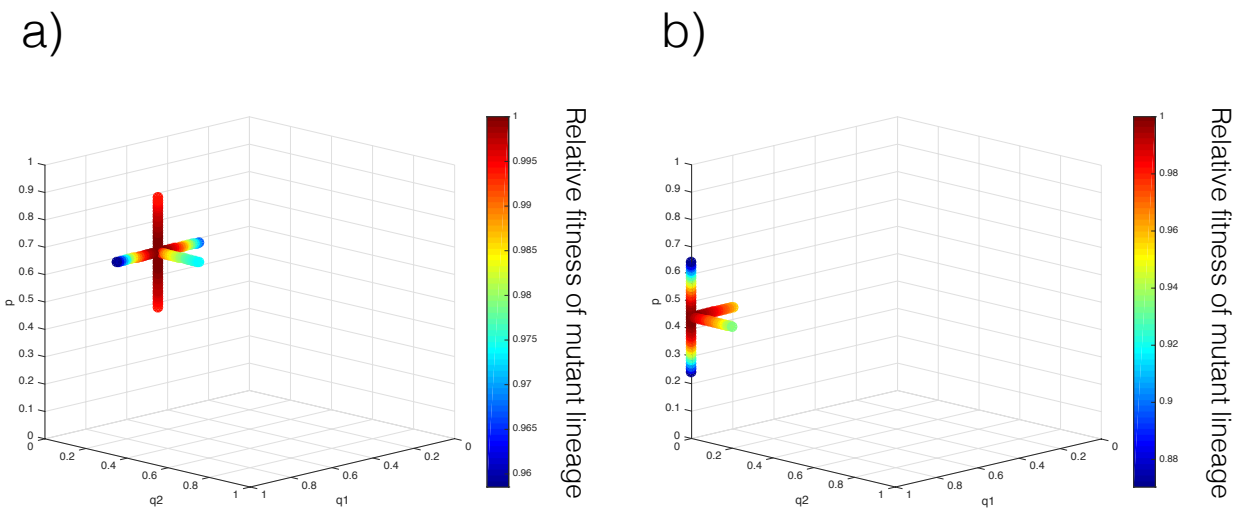
In the following we list the parameter values used to generate Figure 5. The results for these specific parameter values are representative of the broader pattern observed (not presented). The effect of the shape of the return (α; Figures 5a and 5c): $R = 1/3$, $e = 0.9$, $\lambda = 0.505$ (helper reproductives) and $\lambda = 1$ (sterile helpers). The effect of the sociality of the trait (λ; Figures 5b and 5d): $R = 1/3$, $e = 0.9$, $\alpha = 2.2255$ (helper reproductives) and $\alpha = 7.3891$ (sterile helpers).

7.5 Conflicting results with a previous model

Olejars et al. constructed a population genetic model for the invasion of an allele for helper sterility ($q_1 = 1$) in a haplodiploid species[30]. In contrast to both our findings and empirical studies, their analysis found that, in some ecological conditions, queen promiscuity (lower R) actually promotes the genetic invasion of worker sterility (sterile helper and pure reproductive; $q_1 = 1, q_2 = 0$). Davies and Gardner have generalized this model to show that these findings arise for two reasons[6]. First, the analysis only considered sterility alleles that are always expressed by workers ($p \approx 1$). As such, the rarity of the gene and haplodiploid genetics means that half the workers in a monogamous colony will be sterile whereas only a quarter of workers will be sterile in a twice mated colony. If the parameters of the model are tuned such that the efficiency of a colony with 50 percent sterile workers is relatively low compared to that of a colony with 25 percent sterile workers, then worker sterility ($q_1 = 1$) may be more likely to spread in a population of twice mated colonies (lower R) then under monogamous mating (higher R). In contrast, Davies and Gardner found that queen monogamy (higher R) always favours the invasion of a worker sterility gene ($q_1 = 1$) if it is not unconditionally expressed ($0 < p < 1$.)

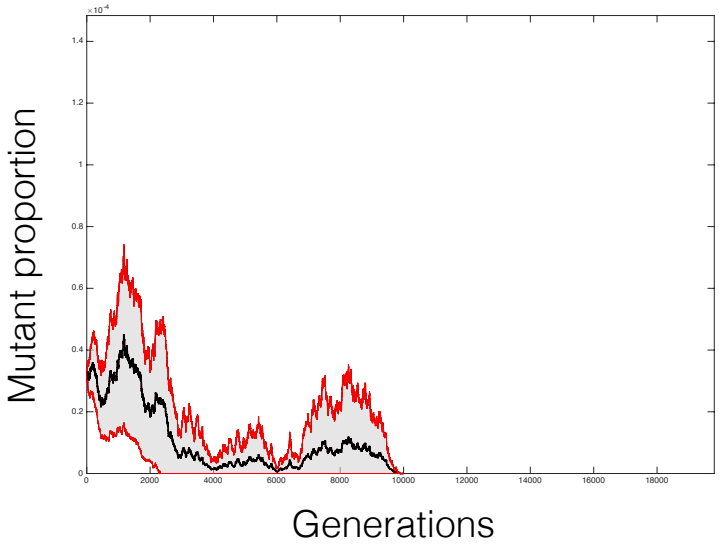
Secondly, Olejars et al. only performed an invasion analysis and did not further investigate the evolutionary outcome after successful invasion (an equilibrium analysis.) Davies and Gardner found that an equilibrium analysis of the model reveals that queen monogamy (higher R) always promotes worker sterility ($q_1 = 1$), as corroborated by our

results[6,30].



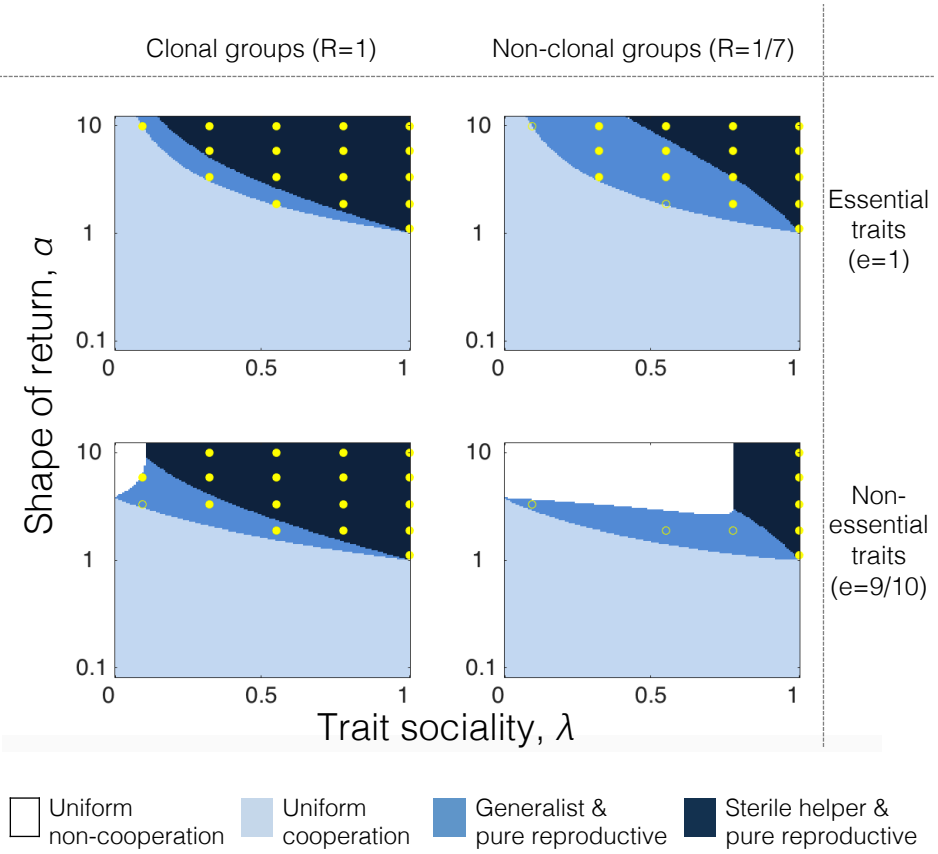
Supplementary figure 1: numerical check of uninvadability

We showed that all of the equilibria in our model are uninvadable and thus ESSs of the system using a novel analytical methodology. In order to corroborate this, we can show numerically that all of the division of labour equilibria are maxima of the local fitness landscape. Above are two division of labour equilibria: a) a generalist and pure reproductive equilibrium (parameters: $\alpha = 1.3956$, $\lambda = 0.703$, $R = 1/3$, $e = 0.9$) and b) a sterile helper and pure reproductive equilibrium (parameters: $\alpha = 1.1052$, $\lambda = 1$, $R = 1$, $e = 0.9$). In both cases, we plot the relative fitness of rare mutant lineages with respect to each of the traits at fixed distances from the equilibrium strategy. We see that in all cases, the equilibrium strategy (at the meeting point of the considered mutant lineages) is the maximum of the local fitness landscape and is thus an ESS. This results is found for all division of labour equilibria considered in the analysis.



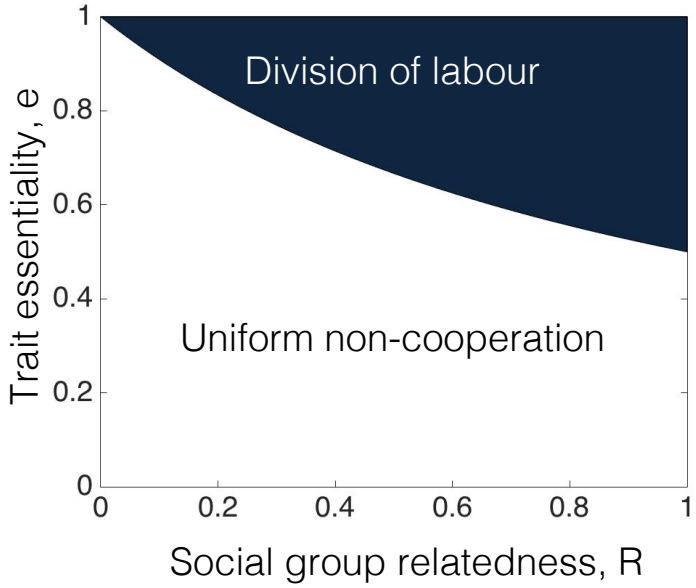
Supplementary figure 2: mutant invasion simulations

We also used individual based-simulations to show that division of labour equilibria are stable under a dynamic process. We show here the results of $10,000$ simulated invasions of a sterile helper and pure reproductive equilibrium (parameters: $\alpha = 9.9742$, $\lambda = 1$, $R = 1/3$, $e = 1$; traits: $p = 0.25$; $q_1 = 1$; $q_2 = 0$) by a mutant lineage with perturbed phenotype probability ($p' = 0.2$). We plot the mean (black line) and 95 percent confidence intervals (red lines) of mutant proportion in the population over time. We see that at the end of the simulation, the upper boundary of the confidence interval is 0 and therefore all mutant lineages have gone extinct. The number of social groups was $M = 10,000$. This invasion analysis was repeated for 60 division of labour equilibria and for perturbations along all possible trait axes.



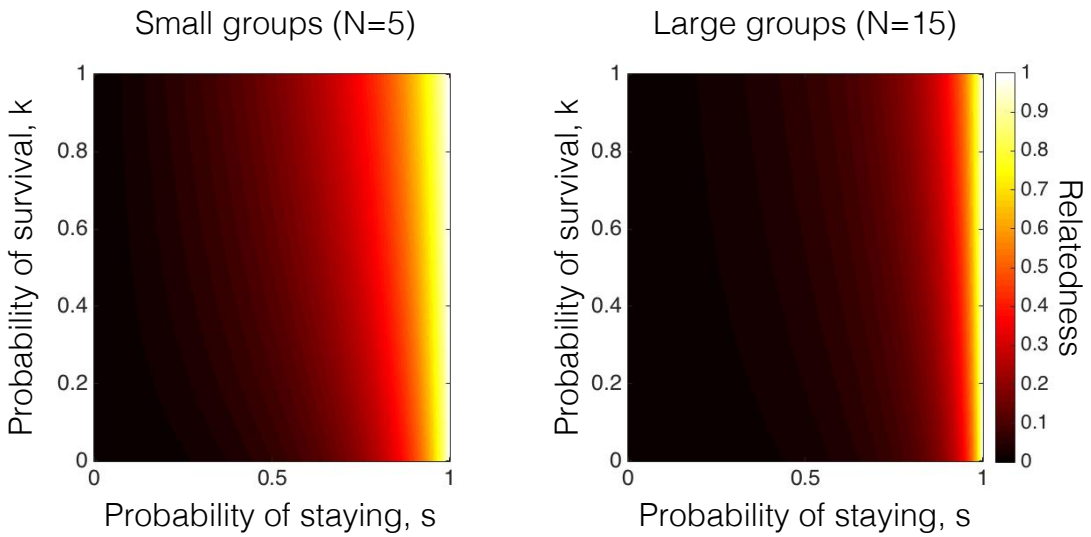
Supplementary figure 3: the stability of division of labour

We used individual-based simulations to show that 60 division of labour equilibria are stable under a dynamic process. For each equilibrium, we considered mutant lineages that deviated by an amount $\pm\epsilon = 0.05$ in each possible trait value. Each such mutant invasion was simulated $L = 10,000$ times for $T = 10,000$ generations in a population consisting of $M = 10,000$ social groups. The yellow circles show the parameter space values of each of the division of labour equilibria that were considered. A filled circle signifies that all mutant lineages went extinct for that equilibrium. An unfilled circle signifies that $1 - 2$ mutant lineages did not go extinct (out of $10,000$) for at least one of the trait perturbations. We note that no mutant lineage in any of the simulations invaded to fixation and that the unfilled circles are still ESSs albeit weakly uninvadable.



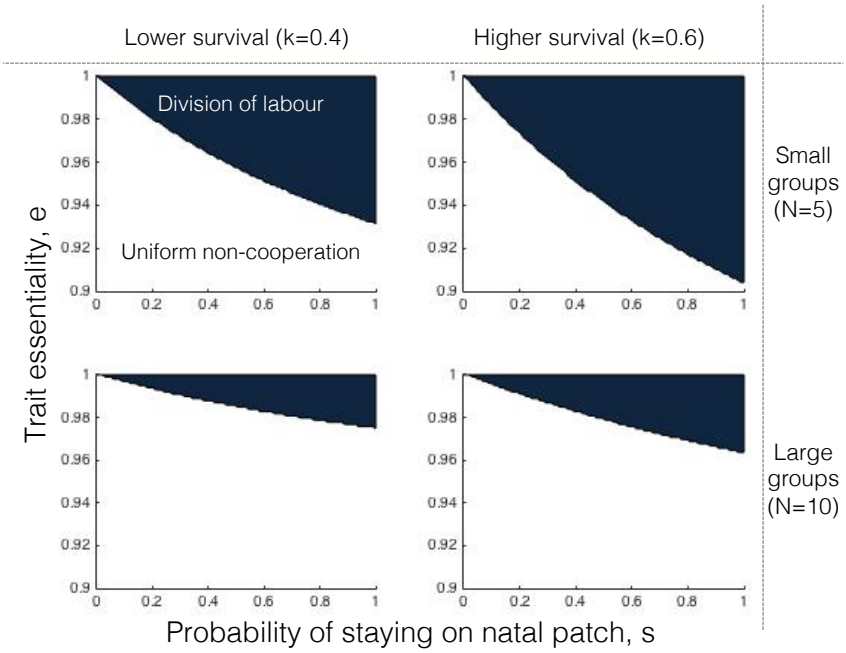
Supplementary figure 4: open model result

In our open model analysis, in which relatedness, R is left as a tuneable, independent parameter, we found that division of labour between a sterile helper ($q_1 = 1$) and a pure reproductive ($q_2 = 0$) is favoured if a threshold condition in trait essentiality is satisfied ($e > 1/(1 + \lambda R)$). Here we depict this threshold condition for $\lambda = 1$ (others-only trait). This is intended for comparison with a closed model analysis.



Supplementary figure 5: the demographic parameters that determine relatedness

In our closed model analysis, relatedness is generated by the interaction of two processes. First, limited dispersal means that a fraction of offspring (s) stay and compete for niches on the natal patch (focal social group.) Secondly, a fraction of individuals (k) survive from one generation to the next leading to overlapping generations. Group size (N) is modelled explicitly due to its interaction with the demographic processes. Here, we depict how each of these terms in our model impact others-only relatedness. We find that limited dispersal (high s), overlapping generations (high k) and small group sizes (low N) lead to high social group relatedness.



Supplementary figure 6: closed model result

For our closed model, we recover the threshold condition in trait essentiality (e) found in our open model analysis (Supplementary figure 4.) We have that division of labour between a sterile helper and pure reproductive is favoured under limited dispersal (high s), overlapping generations (high k) and small group sizes (low N), which are all factors that lead to high relatedness (Supplementary figure 5.)

Previous models	System	Within-group conflict?	All forms of division allowed?	Conditions for division?
Hamilton (1964); Trivers and Hare (1976) and others.	Social insects	✓	✗	✓*
Oster and Wilson (1978); Wilson (1968, 1976) and others.	Social insects	✗	✗	✗**
Michod et al. (2006)	Cell groups	✗	✓	✓
Ackermann et al. (2008)	Cell groups	✓	✗	✓
Willensdorfer (2009)	Cell groups	✗	✗	✓
Rossetti et al. (2010)	Cell groups	✓	✗	✓
Ispolatov et al. (2011)	Cell groups	✗†	✗	✓
Solari et al. (2013)	Cell groups	✗	✗	✓
Tannenbaum (2007)	General	✗	✗	✓
Gavrilets (2010)	General	✗††	✓	✓
Rueffler et al. (2012)	General	✗	✓	✓

Supplementary table 1: **Summary of previous theoretical models of division of labour.** We list the biological system that each model was based upon, inspired by, or applied to. We specify whether each model allows for the potential of within-group conflict. We also include whether the model is free to predict all forms of division of labour (Figure 1) or whether there are any built-in constraints in this respect. Finally, we state whether the model makes explicit predictions for the conditions that favour division of labour. *The conditions required for the evolution of sterile helpers in the social insects has been examined with a number of techniques, including Hamilton's rule, inclusive fitness theory and population genetics. Here we cite the earliest theoretical works. However, this has been a very productive field of research with a large body of literature as the past 5 years alone can attest[2,3,7,12,24,29,30,33,34]. **These models of division of labour in social insects focus on the conditions that favour multiple worker castes and the ratios thereof. †This is an aggregation model and so within-group conflict is allowed in principle. However, the model is solved by assuming perfect asymmetry between partners that could only arise through pleiotropy on the same genotype. ††Social groups are formed by a unicellular bottleneck but genetic variation within groups may arise due to mutations. Thus, while there is scope for within-group conflict, it will tend to be very small.

References

[1] Ackermann, M., Stecher, B., Freed, N. E., Songhet, P., Hardt, W.-D. and Doebeli, M. 2008, 'Self-destructive cooperation mediated by phenotypic noise', *Nature* **454**(7207), 987–990.

[2] Alpedrinha, J., West, S. A. and Gardner, A. 2013, 'Haplodiploidy and the evolution of eusociality: worker reproduction', *The American Naturalist* **182**(4), 421–438.

[3] Avila, P. and Fromhage, L. 2015, 'No synergy needed: ecological constraints favor the evolution of eusociality', *The American Naturalist* **186**(1), 31–40.

[4] Bonner, J. T. 2015, *Cellular Slime Molds*, Princeton University Press.

[5] Brown, S. P. and Taylor, P. D. 2010, 'Joint evolution of multiple social traits: a kin selection analysis', *Proceedings of the Royal Society of London B: Biological Sciences* **277**(1680), 415–422.

[6] Davies, N. G. and Gardner, A. 2016, 'Monogamy promotes worker sterility in insect societies', *bioRxiv* p. 059154.

[7] Davies, N., Krebs, J. and West, S. 2012, 'An introduction to behavioural ecology 4th edition'.

[8] Day, T. and Taylor, P. D. 1998, 'Unifying genetic and game theoretic models of kin selection for continuous traits', *Journal of Theoretical Biology* **194**(3), 391–407.

[9] Eshel, I. and Motro, U. 1981, 'Kin selection and strong evolutionary stability of mutual help', *Theoretical population biology* **19**(3), 420–433.

[10] Fisher, R. M., Cornwallis, C. K. and West, S. A. 2013, 'Group formation, relatedness, and the evolution of multicellularity', *Current Biology* **23**(12), 1120–1125.

[11] Foster, W. 1990, 'Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid pemphigus spyrothecae (hemiptera: Pemphigidae)', *Behavioral Ecology and Sociobiology* **27**(6), 421–430.

[12] Gardner, A., Alpedrinha, J. and West, S. A. 2011, 'Haplodiploidy and the evolution of eusociality: split sex ratios', *The American Naturalist* **179**(2), 240–256.

[13] Gavrilets, S. 2010, 'Rapid transition towards the division of labor via evolution of developmental plasticity', *PLoS Comput Biol* **6**(6), e1000805.

[14] Geritz, S. A., Mesze, G., Metz, J. A. et al. 1998, 'Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree', *Evolutionary ecology* **12**(1), 35–57.

[15] Gordon, D. M. 1996, 'The organization of work in social insect colonies', *Nature* **380**(6570), 121–124.

[16] Griffin, A. S., West, S. A. and Buckling, A. 2004, 'Cooperation and competition in pathogenic bacteria', *Nature* **430**(7003), 1024–1027.

[17] Hamilton, W. D. 1964, 'The genetical evolution of social behaviour. i and ii', *Journal of theoretical biology* **7**(1), 1–52.

[18] Ispolatov, I., Ackermann, M. and Doebeli, M. 2011, 'Division of labour and the evolution of multicellularity', *Proceedings of the Royal Society of London B: Biological Sciences* p. rspb20111999.

[19] Jiricny, N., Diggle, S., West, S., Evans, B., Ballantyne, G., Ross-Gillespie, A. and Griffin, A. 2010, 'Fitness correlates with the extent of cheating in a bacterium', *Journal of evolutionary biology* **23**(4), 738–747.

[20] Koufopanou, V. 1994, 'The evolution of soma in the volvocales', *American Naturalist* pp. 907–931.

[21] Koufopanou, V. and Bell, G. 1993, 'Soma and germ: an experimental approach using volvox', *Proceedings of the Royal Society of London B: Biological Sciences* **254**(1340), 107–113.

[22] Kümmerli, R., Jiricny, N., Clarke, L., West, S. and Griffin, A. 2009, 'Phenotypic plasticity of a cooperative behaviour in bacteria', *Journal of evolutionary biology* **22**(3), 589–598.

[23] Levin, S. R. and West, S. A. 2017, The evolution of cooperation in simple molecular replicators, *in* 'Proc. R. Soc. B', Vol. 284, The Royal Society, p. 20171967.

[24] Liao, X., Rong, S. and Queller, D. C. 2015, 'Relatedness, conflict, and the evolution of eusociality', *PLoS biology* **13**(3), e1002098.

[25] Maynard Smith, J. 1982, *Evolution and the Theory of Games*, Cambridge university press.

[26] Maynard Smith, J. and Price, G. R. 1973, 'The logic of animal conflict', *Nature* **246**(5427), 15–18.

[27] Michod, R. E., Viossat, Y., Solari, C. A., Hurand, M. and Nedelcu, A. M. 2006, 'Life-history evolution and the origin of multicellularity', *Journal of theoretical Biology* **239**(2), 257–272.

[28] Mullon, C., Keller, L. and Lehmann, L. 2016, 'Evolutionary stability of jointly evolving traits in subdivided populations', *The American Naturalist* **188**(2), 175–195.

[29] Nowak, M. A. and Allen, B. 2015, 'Inclusive fitness theorizing invokes phenomena that are not relevant for the evolution of eusociality', *PLoS biology* **13**(4), e1002134.

[30] Olejarz, J. W., Allen, B., Veller, C. and Nowak, M. A. 2015, 'The evolution of non-reproductive workers in insect colonies with haplodiploid genetics', *Elife* **4**, e08918.

[31] Oster, G. F. and Wilson, E. O. 1978, *Caste and ecology in the social insects*, Princeton University Press.

[32] Queller, D. C., Strassmann, J. E. et al. 2014, 'Fruiting bodies of the social amoeba dictyostelium discoideum increase spore transport by drosophila', *BMC evolutionary biology* **14**(1), 105.

[33] Rautiala, P., Helanterä, H. and Puurtinen, M. 2014, 'Unmatedness promotes the evolution of helping more in diplodiploids than in haplodiploids', *The American Naturalist* **184**(3), 318–325.

[34] Rautiala, P., Helanterä, H. and Puurtinen, M. 2017, 'The evolutionary dynamics of adaptive virginity, sex-allocation, and altruistic helping in haplodiploid animals', *Evolution* .

[35] Rossetti, V., Schirrmeister, B. E., Bernasconi, M. V. and Bagheri, H. C. 2010, 'The evolutionary path to terminal differentiation and division of labor in cyanobacteria', *Journal of Theoretical Biology* **262**(1), 23–34.

[36] Rueffler, C., Hermisson, J. and Wagner, G. P. 2012, 'Evolution of functional specialization and division of labor', *Proceedings of the National Academy of Sciences* **109**(6), E326–E335.

[37] Rumbaugh, K. P., Diggle, S. P., Watters, C. M., Ross-Gillespie, A., Griffin, A. S. and West, S. A. 2009, 'Quorum sensing and the social evolution of bacterial virulence', *Current Biology* **19**(4), 341–345.

[38] Sandoz, K. M., Mitzimberg, S. M. and Schuster, M. 2007, 'Social cheating in pseudomonas aeruginosa quorum sensing', *Proceedings of the National Academy of Sciences* **104**(40), 15876–15881.

[39] Solari, C. A., Kessler, J. O. and Goldstein, R. E. 2013, 'A general allometric and life-history model for cellular differentiation in the transition to multicellularity', *The American Naturalist* **181**(3), 369–380.

[40] Strassmann, J. E. and Queller, D. C. 2011, 'Evolution of cooperation and control of cheating in a social microbe', *Proceedings of the National Academy of Sciences* **108**(Supplement 2), 10855–10862.

[41] Strassmann, J. E., Zhu, Y. and Queller, D. C. 2000, 'Altruism and social cheating in the social amoeba dictyostelium discoideum', *Nature* **408**(6815), 965–967.

[42] Tannenbaum, E. 2007, 'When does division of labor lead to increased system output?', *Journal of theoretical biology* **247**(3), 413–425.

[43] Taylor, P. D. 1989, 'Evolutionary stability in one-parameter models under weak selection', *Theoretical Population Biology* **36**(2), 125–143.

[44] Taylor, P. D. 1992, 'Inclusive fitness in a homogeneous environment', *Proceedings of the Royal Society of London B: Biological Sciences* **249**(1326), 299–302.

[45] Taylor, P. D. and Frank, S. A. 1996, 'How to make a kin selection model', *Journal of Theoretical Biology* **180**(1), 27–37.

[46] Taylor, P. D. and Irwin, A. J. 2000, 'Overlapping generations can promote altruistic behavior', *Evolution* **54**(4), 1135–1141.

[47] Trivers, R. L. and Hare, H. 1976, 'Haploidploidy and the evolution of the social insect', *Science* **191**(4224), 249–263.

[48] Veening, J.-W., Igoshin, O. A., Eijlander, R. T., Nijland, R., Hamoen, L. W. and Kuipers, O. P. 2008, 'Transient heterogeneity in extracellular protease production by bacillus subtilis', *Molecular systems biology* **4**(1), 184.

[49] Votaw, H. R. and Ostrowski, E. 2017, 'Stalk size and altruism investment within and among populations of the social amoeba', *Journal of Evolutionary Biology* .

[50] West, S. A. and Cooper, G. A. 2016, 'Division of labour in microorganisms: an evolutionary perspective', *Nature Reviews Microbiology* **14**(11), 716.

[51] West, S. A., Diggle, S. P., Buckling, A., Gardner, A. and Griffin, A. S. 2007, 'The social lives of microbes', *Annual Review of Ecology, Evolution, and Systematics* pp. 53–77.

[52] Willensdorfer, M. 2009, 'On the evolution of differentiated multicellularity', *Evolution* **63**(2), 306–323.

[53] Wilson, E. O. 1968, 'The ergonomics of caste in the social insects', *The American Naturalist* **102**(923), 41–66.

[54] Wilson, E. O. 1976, 'Behavioral discretization and the number of castes in an ant species', *Behavioral Ecology and Sociobiology* **1**(2), 141–154.

[55] Wilson, E. O. 1984, 'The relation between caste ratios and division of labor in the ant genus pheidole (hymenoptera: Formicidae)', *Behavioral Ecology and Sociobiology* **16**(1), 89–98.

Previous models	**System**	α	R	$\lambda^{\dagger}$	e	**Form(s) of division**	**Condition for division:**
Michod et al. (2006)	Volvocine algae	-	1	$\frac{N-1}{N}$	1	all	if returns accelerating ($\alpha > 1$)
Tannenbaum (2007)	General	> 1	1	$\frac{N-1}{N}$	1	only sterile helper and pure reproductive	No conditions relevant here
Ackermann et al. (2008)	*Salmonella typhimurium*	1	-	1	-	only sterile helper and pure reproductive	if high relatedness (R)
Willensdorfer (2009)	Volvocine algae, *D. discoideum*	-	1	$\frac{N-1}{N}$	1	assumes helper is sterile	for many shapes of return (α)
Rossetti et al. (2010)	Cyanobacteria	-	0 or 1	$\frac{N-1}{N}$	-	only sterile helper and pure reproductive	only if $R = 1$
Gavrilets (2010)	General	-	≈ 1	$\frac{N-1}{N}$	1	all	if α is high
Ispolatov et al. (2011)	Volvocine algae, cyanobacteria	-	1	$\frac{N-1}{N}$	1	not specified	if returns accelerating ($\alpha > 1$)
Rueffler et al. (2012)	General	-	1	$\frac{N-1}{N}$	-	not specified	if returns accelerating ($\alpha > 1$)
Solari et al. (2013)	Volvocine algae	-	1	$\frac{N-1}{N}$	1	assumes helper is sterile	if returns accelerating ($\alpha > 1$)

Supplementary table 2: **Specific links to some previous models of division of labour.** Only the microbial and general models of division of labour have attempted to derive conditions for which division of labour is of adaptive value. We list the biological systems that each model was based upon or inspired by. For ease of comparison we only consider the model assumptions and predictions of each that are relevant to the parameter space of our theoretical model. The parameters listed are: the shape of the non-linear return to cooperation, α; relatedness to social group neighbours, R; the sociality of the trait, λ and how essential the trait is, e. N is the fixed size of the social group and the symbol '$-$' signifies that the parameter (or closest approximation thereof) is left free to vary. We also include whether the model is free to predict all forms of division of labour or whether there are any built-in constraints in this respect. Each model may make further assumptions or include further parameters not captured by our model (i.e. group size, rate of mutation, cost of differentiation) and predictions for how division of labour may depend on these factors is not presented here. † For many of these models, group fitness maximisation is assumed, and so how the benefits of cooperation are shared within the group is not made explicit. For these models, we say that the cooperative trait is 'whole-group' ($\lambda = (N/(N-1))$ as all individuals receive the same benefits from cooperation.

Chapter 3: The evolution of predominantly sterile social groups

Abstract

The division of labour between sterile helpers and pure reproductives is found across the tree of life and at many levels of biological organization, from microbial societies to eusocial insect hives. The percentage of individuals within groups that are sterile helpers can range from very small, in some 20%, to an overwhelming amount close to 100% in multicellular organisms and social insects. Many theoretical models give conflicting predictions for the percentage of sterile helpers that may stably evolve. Some theoretical models predict that the percentage of sterile helpers can never stably exist above 50%. In others, the predicted percentage of sterile helpers can vary to nearly 100%. No work has examined what fundamental assumptions distinguish these two kinds of model. We develop here a series of mathematical models to investigate what kinds of biological assumptions are required for both helpers to stably outnumber reproductives (greater than 50%) and for the evolution of an overwhelming proportion of helpers (nearly 100%). We find that social groups composed of predominantly sterile helpers seem to require mechanisms that cause helpers to provide disproportionate social benefits to reproductives relative to the fitness cost of sterility—for example, if helpers provide directed aid to reproductives or if they collaborate with another to yield synergistic group benefits. While we find that higher relatedness tends to favour a higher percentage of helpers, in some cases low relatedness can still foster the evolution of a sterile helper majority.

Introduction

Division of labour, where cooperating individuals specialise to perform different tasks, is found across the levels of biological complexity, from microbial communities to eusocial

insect societies (Boomsma, 2009; Bourke, 2011). The most common form of division is that between sterile helpers, who sacrifice personal reproduction in order to perform tasks beneficial to the rest of the group, and reproductives, who do not perform those beneficial tasks (Cooper and West, 2018). Examples include the reproductive division of labour between queens and worker castes in eusocial insect societies and between germ and somatic cells in multicellular organisms (Bourke, 2011). In both of these cases, there can be an enormous asymmetry between the number of helpers and reproductives in the group (**Figure 1c**). For example, some humans have on average 450 viable germ cells in their lifetimes but many trillions of sterile somatic cells (Cramer and Xu, 1996; Tortora and Derrickson, 2014). Similarly, some insect societies are composed of only one reproductive queen but can harbour millions of sterile workers (Oster and Wilson, 1978). In other, less studied systems, this asymmetry is less stark (**Figure 1b**) and there may in fact be fewer helpers than reproductives (**Figure 1a**). For example, there are more reproductive spore cells than sterile stalk cells in microbial fruiting bodies (Strassmann, Zhu and Queller, 2000).

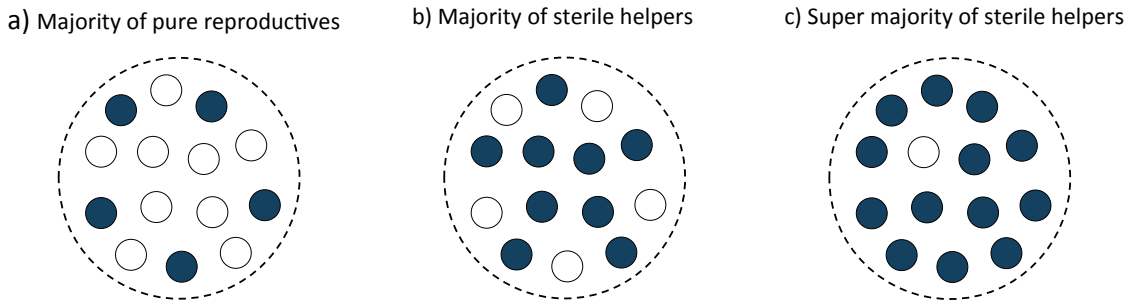


Figure 1: the proportion of sterile helpers. There is variation across the natural world in the proportion of sterile helpers within labour dividing groups. a) In some cases, pure reproductives are more numerous than sterile helpers. b) In other cases, sterile helpers outnumber pure reproductives. c) The most studied labour dividing species (eusocial insect societies and multicellular organisms) are composed of a super-majority of sterile helpers (workers and somatic cells) and only a few pure reproductives (queens and germ cells.)

The many evolutionary models that predict the stable percentage of sterile individuals in a social group make very different predictions. In most models, the percentage of sterile helpers is predicted to always be less than or equal to 50% across all of the parameter space modelled (Michod *et al.*, 2006; Ackermann *et al.*, 2008; Rossetti *et al.*, 2010; Ispolatov, Ackermann and Doebeli, 2012; Cooper and West, 2018). In other models, the percentage of sterile individuals can easily exceed this boundary (more than 50%) (Charlesworth, 1978; Johnstone, 2000; Willensdorfer, 2009) Reproductive skew theory seeks to determine how a few dominant individuals can monopolise reproduction within animal societies (Vehrencamp, 1979; Johnstone, 2000). However, these systems are based on conflict and power dynamics and thus do no constitute the kind of cooperative division of labour that we are interested in here.

In this work, we develop a series of theoretical models to clarify the differences between these many models and to determine what kinds of biological assumptions are required to explain both the evolution of more helpers than reproductives (greater than 50%; **Figure 1b**) and the evolution of a super-majority of helpers, with a minimal number of reproductives and nearly all helpers (close to 100%; **Figure 1c**). We consider four different ways in which the benefits of cooperation could be produced and shared within the group (**Figure 2**). We first consider the production of a diminishable good that disperses across the group and is rivalrously shared by all individuals in the group (**Figure 2a**) (Dionisio and Gordo, 2006). For example, this might correspond to the secretion of finite extracellular factors in some microbial societies that must then be re-absorbed (West and Buckling, 2003; Griffin, West and Buckling, 2004; Frank, 2010). The use of the good by sterile helpers is not translated into reproductive benefits and may be considered a type of ‘soaking’ (Inglis, Hall and Buckling, 2012). We then consider helpers that perform

group-wide actions whose benefits are not diminishable (non-rivalrous) but that are uniformly advantageous to all (**Figure 2b**) (Dionisio and Gordo, 2006). For example, when a meerkat emits a predator warning call, the benefit provided to one group mate does not diminish the benefit to another (Hollén and Radford, 2009). We next consider the possibility that reproductives receive preferential help (Charlesworth, 1978; Willensdorfer, 2009) (**Figure 2c**). For example, social insect workers preferentially care for the queen's brood while actively killing other worker's young (Oster and Wilson, 1978; Bourke, 2011). This is related to the excludability of a trait, whereby not all 'types' of individual may benefit equally from the social actions of a helper (Dionisio and Gordo, 2006; Inglis *et al.*, 2016). Finally, we consider the possibility that helper-helper collaboration leads to synergistic cooperative returns (**Figure 2d**). For example, there may be an accelerating increase in foraging efficiency as the number of foragers in insect societies increases (Oster and Wilson, 1978).

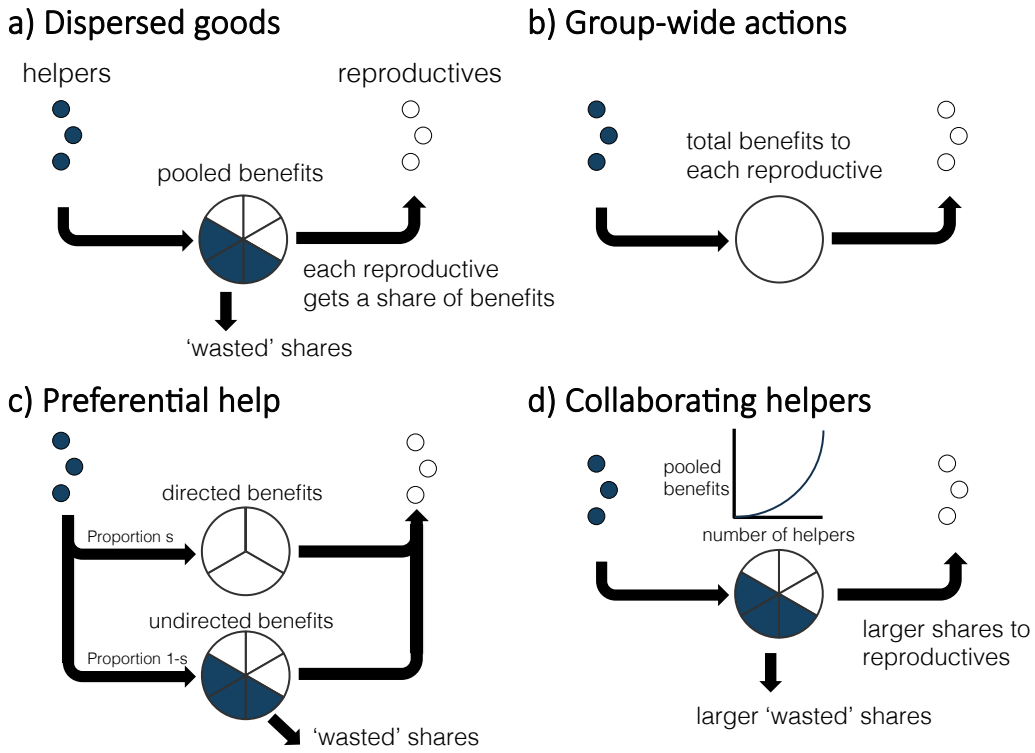


Figure 2: Different possible forms of cooperation. a) Helpers secrete a dispersed diminishable product and reproductives benefit from the local density of the good. As such, the portion of pooled benefits physically surrounding helpers is effectively wasted (soaking). b) Helpers perform group-wide actions whose benefits are not diminishable and needn't be split into shares. Instead, all individuals benefit uniformly from the total benefits produced. c) Reproductives receive preferential help such they receive larger shares of the pooled benefit than those that are wasted by proximity to helpers (or soaked). d) Through combined, synergistic work, a large number of helpers may achieve an accelerating return to social benefits.

In each of the biological models that we construct, we ask: 1) whether a majority of helpers can evolve; 2) if so, what are the conditions that favour more helpers than reproductives; and 3) can a super-majority of helpers arise in some cases.

Model

We deliberately construct simple models in order to focus on the specific details that we believe are of broad relevance. In all scenarios, we assume an infinite population of asexual individuals structured into social groups. Each group is seeded by a finite number of founders, n (haystack model; **Figure 3**). We assume weak selection such that the founders in all groups replicate at equal rates until each group has reached a fixed size, N.

At this point, all reproductives produce a large number of offspring in proportion to their fitness within the group. All grouped individuals then die and the offspring disperse globally to found new groups in the next generation. We define relatedness within groups, R, as the with-replacement probability that two individuals in a group have the same trait value by common descent relative to that of two random individuals from the overall population (in the special case where founders are unrelated, $R = 1/n$; (Grafen, 1985).

Each founder is characterised by a trait value p that determines the relative distribution of its within-group descendants in the distinct roles. A proportion p of its descendants adopt a helper phenotype that does not reproduce but contributes a unit of help to a pool of group benefits (**Figure 3**). Otherwise, a proportion $1 - p$ of its descendants adopt a reproductive phenotype that does not cooperate but benefits from the social good produced in the group. Consequently, each founder faces a tradeoff between spawning more reproductive individuals (lower p) or producing more helpers to increase the fecundity of said reproductives (higher p). The assumption of weak selection means that there is vanishingly small variation (if any) in the trait values p of the group founders (δ-weak selection). In turn, this means that there is negligible selection acting during the growth of groups. Consequently, the group-wide proportion of helpers at the end of the group-growth phase, P, is approximately equal to the average p values of the group founders. We also assume that final group size, *N,* is very large and so we treat helper proportions as continuous traits ($0 < p, P < 1$).

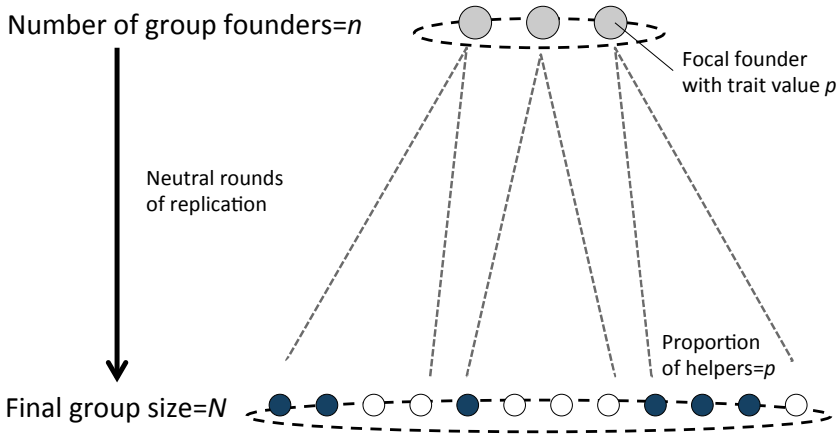


Figure 3: The formation of social groups. We employ a haystack model in which social groups are seeded by $\boldsymbol{n}$ founders, who then replicate with negligible selection until the group has reached a size of $\boldsymbol{N}$ individuals. For a focal founder, the proportion of its within-group descendants that are helpers is equal to its trait value, $\boldsymbol{p}$. The proportion of helpers in the wider group, $\boldsymbol{P}$, is then equal to the average trait value of the social group founders. In the last within-group generation, reproductives produce a large number of offspring proportional to their fitness values evaluated within the group. All grouped individuals then die and the offspring disperse globally to found new social groups at the start of the next group life cycle.

We now consider different possible forms that cooperation may take (**Figure 2**). In each case, we determine the Evolutionary Stable Strategy (ESS) of the proportion of helpers produced by an arbitrary founder, p^* (Maynard Smith and Price, 1973; Parker and Maynard Smith, 1990; Taylor and Frank, 1996). This corresponds to the proportion of helpers within groups that is expected to evolve in the long-term ($P^* = p^*$). In each case, we ask how the different ecological and life-history parameters influence the ESS proportion of helpers (P^*), and in particular if the system can lead to the evolution of more helpers than reproductives ($P^* > 1/2$).

Dispersed goods

We first assume that helpers produce a diminishable product that physically spreads across the social group, such as an extracellular factor in social microbe communities (**Figure**

2a). In this system, reproductives benefit from the local density of the product rather than its sum total. We calculate the local density of the dispersed product as the sum of the pooled benefits, PN, divided by the physical size of the social group, which we assume is equal to group size, N. This gives the following fitness function for an arbitrary group founder with genotype, p:

$$W = (1-p)N\frac{PN}{N}, \qquad (1)$$

where the first term, $(1-p)$N, is the number of reproductive descendants and the second term, (PN/N), is the fecundity of a reproductive in the focal group. In this model, each reproductive receives an equal share of the pooled benefits, and shares going to helpers are `wasted' such as for 'soaking' traits (Inglis, Hall and Buckling, 2012) (**Figure 2a**). Here we make several simplifying assumptions that will be broken in later models. IN particular, we assume that helpers do not provide further aid by recycling or benefiting from the dispersed product in their vicinity. Further, we assume that there are no helper-helper interactions that may lead to greater-than-linear increases in social benefits. In the appendix, we show that the ESS proportion of helpers in this model is:

$$P^* = \frac{R}{1+R}. \qquad (2)$$

Equation 2 shows that: (i) a higher relatedness (higher R), leads to a higher proportion of helpers within social groups (higher P^*; **Figure 4**); and (ii) the maximum proportion of helpers, when $(R = 1)$, is $(P^* = 1/2)$. Consequently, this system cannot lead to the evolution of more helpers than reproductives ($P^* > 1/2$).

The assumption of a dispersed, diminishable good underlies the models of Rosetti *et al.* (2009), Ispolatov *et al.* (2011) and Cooper and West (2018). Accordingly, none of these models could predict the evolution of a majority of helpers ($P^* > 1/2$).

Group-wide actions

We now assume that helpers perform a group-wide task that confers the same, non-diminishable advantage to all individuals in the group (benefits are not depleted or otherwise split into shares) (Dionisio and Gordo, 2006) (**Figure 2b**). Since the social benefits are not partitioned, there are no wasted benefits for this kind of system (no soaking). The fecundity of reproductives is equal to the sum of the pooled benefits in the group (PN), and so the fitness of an arbitrary founder is equal to:

$$W = (1 - p)NPN. \qquad (3)$$

The only difference between this system and a dispersed good (Equation 1) is that the fitness function is rescaled by the size of the social group, N. As such, the indirect fitness benefit of increasing the proportion of group helpers (higher P) is N times bigger than for a dispersed good. However, the direct fitness cost from the increased proportion of sterile descendants (higher p) is *also* N times bigger. As such, both effects cancel and the relative trade-off of increasing the number of helpers over reproductives is identical to that of a dispersed good, resulting in the same ESS value of P^* (Equation 2). Therefore, even at maximal relatedness ($R = 1$), the number of helpers in a group is never predicted to exceed the number of reproductives ($P^* \leq 1/2$; **Figure 4**).

The assumption of non-diminishable, group-wide actions underlies the models of Michod *et al.* (2006) and Ackermann *et al.* (2018). In accordance, neither of these models could predict the evolution of a majority of helpers ($P^* > 1/2$).

In the next two models, we consider key extensions to the dispersed goods model and whether these can lead to a higher proportion of sterile helpers (higher P^*).

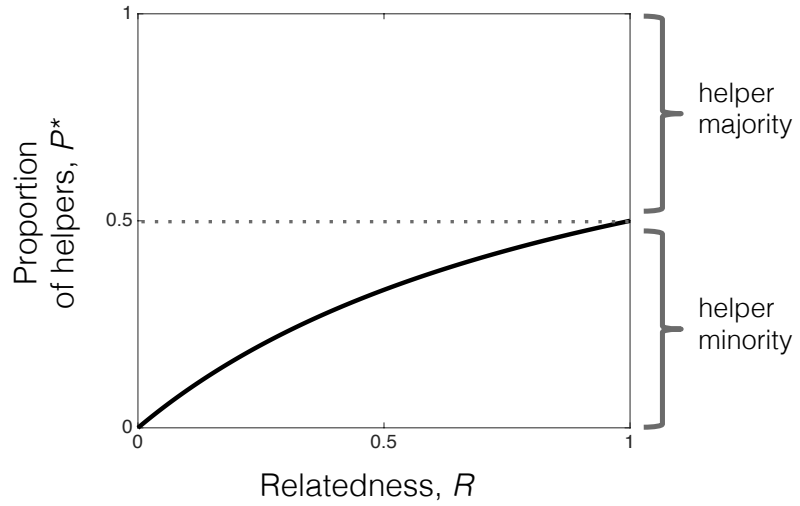


Figure 4: Proportion of helpers for dispersed goods and group-wide actions. We find that dispersed goods and group-wide actions both give the same Evolutionarily Stable Strategy (ESS) for the proportion of helpers, $\boldsymbol{P}^*$. In both cases, an increasing relatedness (higher R) leads to a higher proportion of helpers (higher $\boldsymbol{P}^*$). However, the number of helpers will always be less or equal to the number of reproductives ($\boldsymbol{P}^* \leq \mathbf{1/2}$) and, as such, a helper majority will not be favoured for these simple forms of cooperation.

Preferential help

We consider here the possibility that helpers may actively direct the benefits of their cooperation to reproductives over helpers (**Figure 2a**). This is a particular example of an excludable trait, wherein not all individuals may benefit from cooperation equally (Dionisio and Gordo, 2006; Inglis *et al.*, 2016). In this instance, the size of social shares that benefit reproductives may be larger than those that are wasted by proximity to helpers

(or by soaking) (Inglis, Hall and Buckling, 2012). This contrasts with the non-excludability of the dispersed goods model, in which the size of shares that are wasted (or soaked) are identical to those that benefit reproductives.

Aside from directed help, there are other mechanisms that can lead to larger shares for reproductives than those that are wasted on helpers (helper-excludability). For example, if reproductives are larger than helpers, they may take up a larger proportion of the dispersed good (Willensdorfer, 2009). The same essential effect may arise if helpers actually use the social benefits to boost their own cooperation and provide further help to reproductives. In the appendix, we show that larger helpers and recycled benefits lead to the same qualitative results as for directed help.

Here, we assume that a proportion s of the benefits produced by helpers is directed to and shared only by the group's reproductives (**Figure 2a**). The remaining proportion $(1-s)$ is non-directed just as for dispersed goods (**Figure 2a**). This may correspond to the relative allocation in eusocial insects between helping tasks that benefit only the queen (brood care) and those that benefit the whole hive more equally (foraging, defense). This gives the following fitness equation for a focal founder:

$$W = (1-p)N\left(s\frac{PN}{(1-P)N} + (1-s)\frac{PN}{N}\right) \qquad (4)$$

where the term $s\frac{PN}{(1-P)N}$ corresponds to the fecundity benefits preferentially directed to reproductives and the term $(1-s)\frac{PN}{N}$ corresponds to the benefits that arise from undirected help. We see that the first of these fecundity benefits may get considerably

large as the proportion of helpers increases (higher P) because there are then fewer reproductives (lower $1-P$) to share the directed help with. The key here is that both pools of cooperative benefits are diminishable but that the rivalry over the benefits is between different individuals in each case (reproductives only in the former and all individuals in the latter) (Dionisio and Gordo, 2006). In the appendix, we show that the ESS proportion of helpers is

$$P^* = \begin{cases} \dfrac{1+2R(1-s)-\sqrt{1-4R^2s(1-s)}}{2(1-s)(R+1)} & \text{if s<1,} \\ R & \text{if s=1.} \end{cases} \quad (5)$$

This model predicts that there can be a majority of helpers in the group ($P^* > 1/2$; **Figure 5**). More specifically, the results shows that; (i) a higher relatedness (higher R) and a higher proportion of directed help (higher s) both lead to more helpers (higher P); (ii) if groups are maximally related ($R = 1$), then a marginal amount of directed help ($s > 0$), will lead to more helpers than reproductives ($P^* > 1/2$); and (iii) in maximally related groups ($R = 1$), if more help is directed than undirected ($s > 1/2$), then founders should produce only one reproductive, and otherwise produce helpers (a super majority of helpers; $P^* = \frac{N-1}{N} \approx 1$).

The proposed mechanism underlies the models of Charlesworth (1978) and Willensdorfer (2009). Charlesworth (1978) developed a series of models that employed either a dispersed good assumption ($s = 0$) or total preferential help to reproductives ($s = 1$). In agreement with our results, he found that a majority of helper ($P^* > 1/2$) could never arise for fully dispersed goods ($s = 0$), but could sometimes arise when help is directed ($s = 1$).

Willensdorfer (2009) developed a model in which sterile cells are smaller than reproductive cells ($s > 0$) and found that the proportion of sterile cells could exceed that of germ cells ($P^* > 1/2$).

Collaborating helpers

As a final possibility, we consider a scenario in which efficiency gains from more helper-helper interactions means that twice the number of helpers yields more than twice the social benefits (synergy; **Figure 2d**). We assume that reproductives depend non-linearly on the proportion of helpers in the group, P, such that fecundity may be expressed as P^{α}, where the parameter $\alpha \geq 1$ controls the shape of the synergistic return (not the same α as that contained in Chapter 2). If $\alpha = 1$, then additional helpers provide only a proportional increase in benefits. However, if $\alpha > 1$, then there is an accelerating return from increasing the proportion of helpers (**Figure 2d**). As the shape of the return increases (higher α), there are disproportionately more synergistic benefits from additional helpers. This gives the following expected fitness of a focal individual:

$$W = (1 - p)NP^{\alpha}, \qquad (6)$$

In the appendix, we show that the ESS proportion of helpers is:

$$P^* = \frac{\alpha R}{1+\alpha R}. \qquad (7)$$

Once again, we find that a majority of helpers may evolve ($P^* > 1/2$; **Figure 5**). Equation 10 shows that: (i) a higher relatedness and accelerating return (higher R and α), both lead to a higher proportion of helpers (higher P^*); (ii) when social groups are maximally related

($R = 1$), even a marginal accelerating return ($\alpha > 1$) can lead to more helpers than reproductives ($P^* > 1/2$); (iii) At lower relatedness values (lower R), a sufficiently large accelerating return ($\alpha > 1/R$) can lead to a helper majority ($P^* > 1/2$); and (iv) a super majority of helpers ($P^* = \frac{N-1}{N} \approx 1$) is not likely to evolve as it can only occur in the limit as the shape of synergistic return goes to infinity ($\alpha \to \infty$).

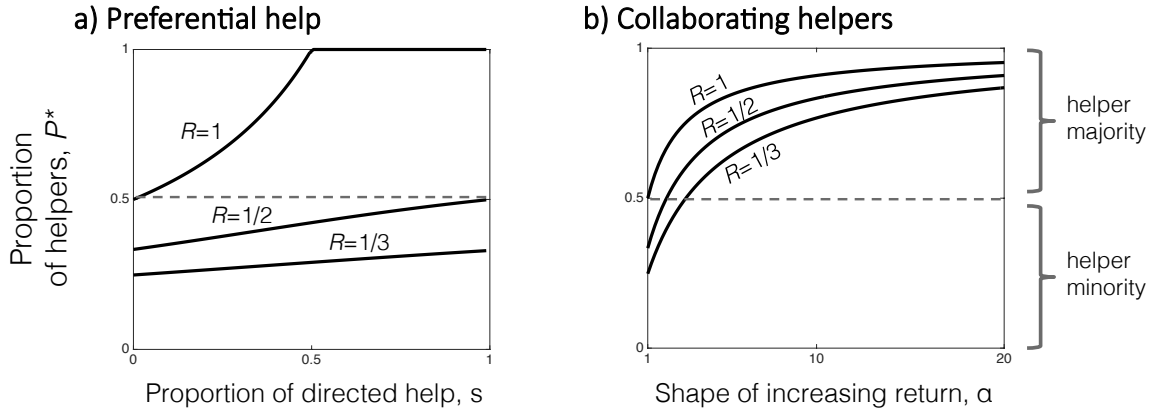


Figure 4: Expected proportion of helpers for preferential help and collaborating helpers. a) If reproductives receive preferential help, we find that a higher relatedness (higher $\boldsymbol{R}$) and a higher proportion of directed help (higher $\boldsymbol{s}$) both lead to more helpers (higher $\boldsymbol{P^*}$). If groups are maximally related ($\boldsymbol{R = 1}$), then even a marginal amount of directed help ($\boldsymbol{s > 0}$), will lead to more helpers than reproductives ($\boldsymbol{P^* > 1/2}$), and if a majority of help is directed ($\boldsymbol{s > 1/2}$), then a maximal number of helpers may arise ($\boldsymbol{P^* = (N-1)/N}$). b) If helpers collaborate, we find that a more accelerating return (higher $\boldsymbol{\alpha}$) and higher relatedness (higher $\boldsymbol{R}$) both lead to a higher expected proportion of helpers (higher $\boldsymbol{P^*}$). If relatedness is maximal ($\boldsymbol{R = 1}$), then even a marginally accelerating return ($\boldsymbol{\alpha > 1}$) leads to a helper majority ($\boldsymbol{P^* > 1/2}$). If relatedness is not maximal ($\boldsymbol{R < 1}$), then a sufficiently large accelerating return ($\boldsymbol{\alpha > 1/R}$) can still produce a majority of helpers ($\boldsymbol{P^* > 1/2}$).

Discussion

We have found that some forms of cooperation can foster the evolution of a helper majority (sometimes $P^* > 1/2$) whereas others cannot (always $P^* \leq 1/2$). If helpers produce a non-excludable, diminishable social good (**Figure 2a**) or if they perform group-wide actions that provide non-excludable and non-diminishable benefits (**Figure 2b**), then helpers are not favoured to outnumber reproductives ($P^* < 1/2$; **Figure 4**). In contrast, if

the cooperative trait is excludable and help is directed such that reproductives receive a larger share of the benefits (**Figure 2c**), or if collaborating helpers yield synergistic benefits (**Figure 2d**), then groups with a majority of sterile helpers may evolve ($P^* > 1/2$; **Figure 5**). We have found that a super majority of helpers ($P^* \approx 1$) is most likely to arise when help is directed predominantly to reproductives (**Figure 5**). Across all systems, we have found that a higher relatedness (higher R) favours a higher proportion of helpers (higher P^*) but that lower relatedness ($R < 1$) can nevertheless still lead to the evolution of a helper majority ($P^* > 1/2$; **Figure 5**).

What key underlying assumption distinguishes the models that can lead to the evolution of a helper majority from those that cannot? From the perspective of a focal founder, the cost of spawning more sterile helpers (higher p) is a proportional decrease in fitness due to fewer reproductive descendants (lower $1 - p$). However, the benefit of spawning more helpers (higher p) is that these individuals may then increase the fecundity of the reproductives that *are* produced.

In both systems that favour a majority of sterile helpers, the production of more helpers (higher p) leads to *disproportionate* gains to the fecundity of reproductives. Amongst other possibilities, these disproportionate gains may arise due to the synergy of increased helper-helper interactions or because more helpers means that reproductives have fewer other individuals to share diminishable reproductive directed benefits with. In contrast, in both systems that do not favour a majority of sterile helpers, the production of more helpers (higher p) only leads to a *proportional* increase in reproductive fecundity. Consequently, it seems that the evolution of a majority of sterile helpers requires that additional helpers

(higher p) somehow provide more bang-for-their-buck returns (greater than linear) than arises in the simplest models.

Can these findings help us to understand patterns in nature? The forms of cooperation that favour a majority of helpers are not mutually exclusive. In fact, many species interact in ways that reflect multiple mechanisms. For example, in eusocial insect societies, sterile workers provide some directed help to the queen's brood, they are morphologically smaller than queens, they benefit from the help of other workers, and they collaborate with one another to produce synergistic benefits for the hive (Oster and Wilson, 1978). Multicellular bodies also employ each of these mechanisms. For example, an unfertilised female egg in humans is larger than all other cells, there are entire organs composed of somatic cells that act only to maintain and aid the population of germ cells, and the intricate functioning of the human body yields returns that are larger than the sum of its parts (Bourke, 2011; Tortora and Derrickson, 2014). It is perhaps not coincidental that both of these cases represent key instances in nature of extreme skew toward a helper super-majority. However, this is not a conclusive test of our predictions and highlights the need for further empirical work cataloguing both the proportion of helpers across labour dividing species and on the particular mechanisms by which helpers provide benefits to reproductives.

Why does any of this matter? Division of labour is predicted to play a key role in the evolution of complex life (Bourke, 2011). When individuals divide labour, this can lead to mutual dependence between partners and complex group adaptations may ensue (Gardner and Grafen, 2009; Bourke, 2011). However, the most complex biological life forms are not simply those that harbour a division between sterile helpers and pure reproductives.

Complex life forms are also defined by the richness and variety of the different kinds of sterile helpers of which they are composed (e.g. the different eusocial insect castes and multicellular cell types) (Bourke, 2011). As such, the evolution of a majority of helpers may be a necessary pre-cursor to the evolution of more sophisticated groups. Consequently, if we want to understand why some forms of life on Earth have become so complex, then we need to understand not just why individuals divide labour but also when this can lead to groups composed of many helpers.

Appendix 1: dispersed goods.

The following is the fitness equation for a focal founder with trait value p in a social group with average trait value P:

$$W = (1 - p)NP \tag{A1}$$

We employ the Taylor-Frank method in order to identify the equilibrium value of this model (Taylor and Frank, 1996). As such we solve for the value p^* such that directional selection in the trait $\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)$ is zero in a monomorphic population ($p = P = p^*$). This gives the following equation:

$$\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)\Big|_{p=P=p^*} = -Np + R(1 - p)N = 0 \tag{A2}$$

Solving Equation A2 for p gives the equilibrium value of the proportion of helpers (Equation 2).

Appendix 2: preferential help.

The following is the fitness equation for a focal founder with trait value p in a social group with average trait value P:

$$W = (1-p)N\left(s\frac{P}{(1-P)} + (1-s)P\right) \tag{A3}$$

W we solve for the value p^* such that directional selection in the trait $\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)$ is zero in a monomorphic population ($p = P = p^*$). This gives the following equation:

$$\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)\Big|_{p=P=p^*} \tag{A4}$$

$$= \frac{N}{1-p}(p^2((R+1)(1-s))$$

$$- p(1 + 2R(1-s)) + R) = 0$$

We solve for p using the quadratic equation to find the equilibrium value given in Equation 5.

Appendix 3. large reproductives.

Let θ be the size of reproductives relative to helpers such that $\theta = 2$ means that reproductives are twice the size of helpers. In this case, the fitness of a focal founder is:

$$W = (1-p)N\left(\frac{P}{P(1/\theta) + (1-P)}\right) \tag{A5}$$

W we solve for the value p^* such that directional selection in the trait $\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)$ is zero in a monomorphic population ($p = P = p^*$). This gives the following equation:

$$\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)\Big|_{p=P=p^*} = \frac{N\theta}{(p+(1-p)\theta)^2}(p^2(\theta-1) - p\theta(1+R) + R\theta) = 0 \tag{A6}$$

We solve for p using the quadratic equation to find that the equilibrium value is:

$$P^* = \begin{cases} \dfrac{R}{R+1} & \text{if } \theta=1, \\ \dfrac{1+R-\sqrt{(1+R)^2 - 4(\theta-1)R/\theta}}{2(\theta-1)/\theta} & \text{if } \theta>1. \end{cases} \tag{A7}$$

We see that this mechanism allows for a majority of helpers in the group ($P^* > 1/2$). Specifically, a higher relatedness (higher R) and larger reproductives (higher θ) both lead to more helpers (higher P^*). If relatedness is maximal ($R = 1$), then even a marginal increase in reproduction size ($\theta > 1$) will lead to a majority of helpers ($P^* > 1/2$).

Appendix 4. recycled benefits.

Assume that helpers may use the social benefits to boost their own cooperative output and thus provide further social benefits to reproductives. In this way, helpers reduce the size of shares that are otherwise wasted in the dispersed goods model. Let us assume that a helper

diminishes the size of its wasted share of social benefits by a fraction ϕ and that the social benefit shares of reproductives are proportionally augmented. We calculate the amount that reproductive fecundity is increased by solving the following equation for x:

$$PNP(1-\phi) + (1-p)NPx = PN \tag{A8}$$

This conservation simply states that the sum of the shares wasted on helpers ($PNP(1-\phi)$) and the sum of shares given to reproductives ($(1-p)NPx$) must equal the total amount of social benefits produced in the group (PN). We find that reproductive fecundity is amplified by the factor:

$$x = 1 + \frac{P\phi}{1-P} \tag{A9}$$

Consequently, this gives the following fitness equation in the scenario where helpers recycle social benefits to boost reproductive fecundity:

$$W = (1-p)NP\left(1 + \frac{P\phi}{1-P}\right) \tag{A10}$$

A simple re-arranging of terms shows that this is equal to the fitness equation for preferential help (Equation A3) where the degree to which helpers recycle shares is equal to the proportion of help directed to reproductives ($\phi = s$).

Appendix 5. collaborating helpers

The following is the fitness equation for a focal founder with trait value p in a social group with average trait value P:

$$W = (1 - p)P^{\alpha} \tag{A11}$$

W we solve for the value p^* such that directional selection in the trait $\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)$ is zero in a monomorphic population ($p = P = p^*$). This gives the following equation:

$$\left(\frac{\partial W}{\partial p} + R\frac{\partial W}{\partial P}\right)\Big|_{p=P=p^*} = p^{\alpha-1}(p(-1 - \alpha R) + \alpha R) = 0 \tag{A12}$$

Solving Equation A12 for p gives the equilibrium value of the proportion of helpers (Equation 10).

Appendix 6. are the ESSs maxima?

In our analysis, we have only shown that the trait values of p are equilibria of the fitness equation. In order to ensure that they are indeed ESSs, we need to show that they are maxima and not minima of the fitness landscape. To do this we use the following condition derived in Cooper and West (2018):

$$\left(\frac{\partial^2 W}{\partial p^2} + 2R\frac{\partial^2 W}{\partial p \partial P} + R^2\frac{\partial^2 W}{\partial P^2}\right)\Big|_{p=P=p^*} < 0 \tag{A13}$$

Using this condition, we find that the equilibrium proportion of helpers in each model is indeed an ESS of the system.

References

Ackermann, M. *et al.* (2008) 'Self-destructive cooperation mediated by phenotypic noise', *Nature*, 454, pp. 987–990. doi: 10.1038/nature07067.

Boomsma, J. J. (2009) 'Lifetime monogamy and the evolution of eusociality', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, pp. 3191–3207. doi: 10.1098/rstb.2009.0101.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Charlesworth, B. (1978) 'Some Models of the Evolution of Altruistic Behaviour between Siblings', 72, pp. 297–319.

Clutton-Brock, T. H. (1998) 'Reproductive skew, concessions and limited control', *Trends in Ecology and Evolution*, 13, pp. 288–292. doi: 10.1016/S0169-5347(98)01402-5.

Cooper, G. A. and West, S. A. (2018) 'Division of labour and the evolution of extreme specialization', *Nature Ecology and Evolution*. Springer US, 2, pp. 1161–1167. doi: 10.1038/s41559-018-0564-9.

Cramer, D. W. and Xu, H. (1996) 'Predicting age at menopause', *Maturitas*, 23, pp. 319–326. doi: 10.1016/0378-5122(96)00992-9.

Dionisio, F. and Gordo, I. (2006) 'The tragedy of the commons , the public goods dilemma , and the meaning of rivalry and excludability in evolutionary biology', *Evolutionary Ecology Research*, 8, pp. 321–332.

Frank, S. A. (2010) 'A general model of the public goods dilemma', *Journal of Evolutionary Biology*, 23, pp. 1245–1250. doi: 10.1111/j.1420-9101.2010.01986.x.

Gardner, A. and Grafen, A. (2009) 'Capturing the superorganism: A formal theory of group adaptation', *Journal of Evolutionary Biology*, 22, pp. 659–671. doi: 10.1111/j.1420-9101.2008.01681.x.

Grafen, A. (1985) 'A geometric view of relatedness', *Oxford Surveys in Evolutionary Biology*, 2, pp. 28–89.

Griffin, A. S., West, S. A. and Buckling, A. (2004) 'Cooperation and competition in pathogenic bacteria', *Nature*, 430, pp. 1024–1027. doi: 10.1038/nature02744.

Hollén, L. I. and Radford, A. N. (2009) 'The development of alarm call behaviour in mammals and birds', *Animal Behaviour*, 78, pp. 791–800. doi: 10.1016/j.anbehav.2009.07.021.

Inglis, R. F. *et al.* (2016) 'Presence of a loner strain maintains cooperation and diversity in well-mixed bacterial communities', *Proceedings of the Royal Society B: Biological Sciences*, 283, p. 2682.

Inglis, R. F., Hall, A. R. and Buckling, A. (2012) 'The role of " soaking " in spiteful toxin production in Pseudomonas aeruginosa', *Biology letters*, pp. 3–6.

Ispolatov, I., Ackermann, M. and Doebeli, M. (2012) 'Division of labour and the evolution of multicellularity', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 1768–1776. doi: 10.1098/rspb.2011.1999.

Johnstone, R. A. (2000) 'Models of reproductive skew: A review and synthesis', *Ethology*, 106, pp. 5–26. doi: 10.1046/j.1439-0310.2000.00529.x.

Maynard Smith, J. and Price, G. R. (1973) 'The logic of animal conflict', *Nature*, 246, pp. 15–18. doi: 10.1038/246015a0.

Michod, R. E. *et al.* (2006) 'Life-history evolution and the origin of multicellularity', *Journal of Theoretical Biology*, 239, pp. 257–272. doi: 10.1016/j.jtbi.2005.08.043.

Oster, G. F. and Wilson, E. O. (1978) *Caste and ecology in the social insects*, *Caste and ecology in the social insects*. Princeton: Princeton University Press.

Parker, G. A. and Maynard Smith, J. (1990) 'Optimality theory in evolutionary biology',

Nature, 348, pp. 27–33. doi: 10.1038/348027a0.
Rossetti, V. *et al.* (2010) 'The evolutionary path to terminal differentiation and division of labor in cyanobacteria', *Journal of Theoretical Biology*, 262, pp. 23–24. doi: 10.1016/j.jtbi.2009.09.009.
Taylor, P. D. and Frank, S. A. (1996) 'How to make a kin selection model', *Journal of Theoretical Biology*, 180, pp. 27–37. doi: 10.1006/jtbi.1996.0075.
Tortora, G. J. and Derrickson, B. (2014) *Principles of Anatomy and Physiology*, *Wiley*. United States of America: John Wiley & Sons. doi: 10.1016/S0031-9406(05)60992-3.
Vehrencamp, S. L. (1979) 'The Roles of Individual, Kin, and Group Selection in the Evolution of Sociality', in *Social behavior and communication*. Boston, MA: Spriner, pp. 351–394.
West, S. A. and Buckling, A. (2003) 'Cooperation, virulence and siderophore production in bacterial parasites', *Proceedings of the Royal Society B: Biological Sciences*, 270, pp. 37–44. doi: 10.1098/rspb.2002.2209.
Willensdorfer, M. (2009) 'On the evolution of differentiated multicellularity', *Evolution*, 63, pp. 306–323. doi: 10.1111/j.1558-5646.2008.00541.x.

Chapter 4: The evolution of coordinated division of labour

Abstract

Division of labour occurs when cooperating individuals specialise on distinct tasks. Researchers of biological division of labour can generally be divided into two broad camps: those who ask evolutionary questions about why individuals should be favoured to divide labour; and those who ask mechanistic questions about how individuals actually specialise into their respective roles. Here, we bridge that gap by asking an evolutionary question about the mechanisms underlying individual specialisation. Namely, why do individuals in some labour-dividing species adopt their respective roles using environmental information whereas other species divide labour by purely random specialisation? We develop a mathematical model to determine: (1) whether there exists an explanation for these different mechanisms rooted in natural selection; and, if so, (2) what conditions favour one mechanism over the other? We find that the mechanism of division of labour can in fact be explained with natural selection and so may constitute instances of adaptation rather than historical accident or ecological constraint. We show generally that the use of environmental information, allowing for a coordinated division of labour, is most likely to occur in small groups and where cooperation is essential for survival and reproduction, or in larger species where the cost of using environmental information is negligible relative to the metabolic expenditure of everyday life.

Introduction

Division of labour, where cooperating individuals specialise to perform different tasks, occurs at all levels of biological complexity, from microbial communities to human societies (Boomsma, 2009; Bourke, 2011). Individual specialisation into a particular role

can be achieved through at least three different mechanisms: the use of environmental information, by genetic differences between individuals, or through random specialisation (stochastic noise) (Wahl, 2002; Schwander *et al.*, 2010; Ackermann, 2015; West and Cooper, 2016). The use of environmental information will tend to occur by social signals or cues (Maynard Smith and Harper, 2003). In different social insect species, whether females become queens or workers can be determined anywhere along a continuum, from purely environmental signals, depending upon the food they are fed by other workers, to largely genetic, depending upon their genotype (Schwander *et al.*, 2010) In some microbes, such as cyanobacteria filaments, labour is divided using environmental cues (fixed nitrogen concentration gradient) whereas in others, such as *Salmonella enterica* pathogens, cells commit to alternate phenotypes randomly, depending upon unpredictable biochemical fluctuations within each cell (noise) (Ackermann *et al.*, 2008; Davidson and Surette, 2008; Veening *et al.*, 2008; Veening, Smits and Kuipers, 2008; Rossetti *et al.*, 2010).

We lack evolutionary explanations for why these different mechanisms are used to produce division of labour in different species (Wahl, 2002). Most previous work has tended to be either mechanistic, focusing on how different phenotypes are produced (caste determination), or evolutionary, focusing on why division of labour is favoured (Oster and Wilson, 1978; Michod, 2006; Gavrilets, 2010; Ispolatov, Ackermann and Doebeli, 2012; Cooper and West, 2018). Consequently, we do not understand why different mechanisms have evolved in each case (Wahl, 2002). For example, why does division of labour arise by random specialisation in some microbial species, but is mediated by environmental information in others? Why is there a conspicuous dearth of random caste determination in animal societies?

Here, we ask when natural selection should favour division of labour using environmental information over division of labour by random specialisation (**Figure 1b**). We focus on these in particular because they both occur in bacteria and other microorganisms, and so both mechanisms are viable strategies at a common scale of biological organisation. Our hypothesis is that the use of environmental information can allow for a more precise coordination of labour between individuals in a group. Although the use of signals or cues could incur a metabolic cost (emission or sensing), the mechanism may nevertheless be favoured if a precise allocation of labour is particularly advantageous. For example, in small groups, there may be strong selection to ensure that all phenotypes are represented in the group at the optimal ratio (**Figure 1b**) (West and Cooper, 2016).

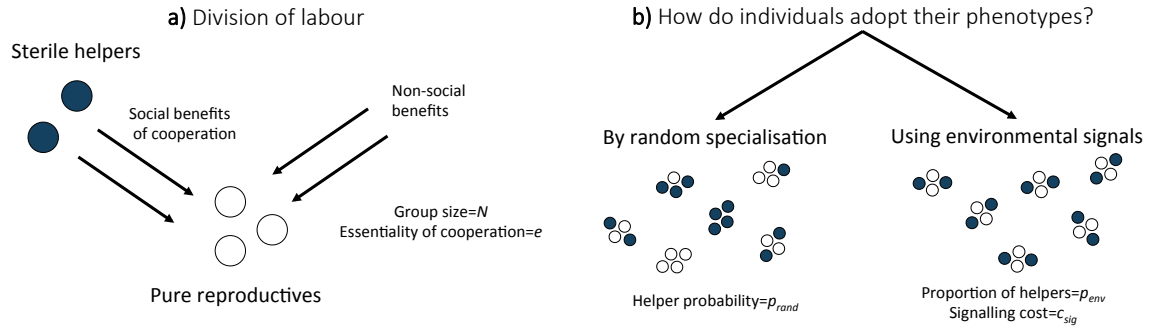


Figure 1. How is labour divided? a) Within each group, labour is divided between sterile helpers who perform cooperative actions beneficial to all and pure reproductives who marshal all of their resources for personal fitness. The essentiality of cooperation, *e*, captures the degree to which the fitness of a pure reproductive depends on the social benefits of cooperation rather than on a baseline, non-social benefit from the environment. Each group is composed of *N* individuals. b) We would like to know why some species divide into helper and reproductive roles by random specialisation whereas others use information about the social environment to coordinate labour. When dividing randomly, each individual may become a helper with probability p_{rand} leading to all possible group compositions. When specialising by environmental information, each individual pays a fitness scaling cost, c_{env} (signalling, sensing, or coordination cost), and the resulting groups are all composed of a precise proportion of helpers, p_{env}.

Model

We consider a simple mathematical model, focusing only on the details that we believe are of broad relevance. We consider two sets of clonal groups of fixed group size, *N*, where one set of groups divide labour through random specialisation and the other coordinates labour using environmental information. We then evaluate the relative fitness of each strategy to determine which mechanism will be favoured by natural selection. In each case, we consider the division of labour between sterile helpers that perform a cooperative action beneficial to all and pure reproductives that marshal all of their resources for asexual reproduction (**Figure 1a**). We vary the importance of cooperation with the parameter, e, which determines the extent to which the fecundity of a reproductive depends upon the proportion of helpers in the group, p. This gives the fitness of an arbitrary social group as its number of reproductives $(1-p)N$, multiplied by the sum of the baseline fecundity of reproductives, $(1-e)$, and the increased fecundity due to cooperation in the group, ep.

$$W_0(p) = (1-p)N((1-e)+ep). \qquad (1)$$

This equation illustrates that, within each group, there is an inherent trade-off between the proportion of reproductives in the group $(1-p)$ and the proportion of helpers (p) present to aid those reproductives. Here we have assumed that the fitness of reproductives depends linearly on the proportion of helpers in the group.

Division of labour by random specialisation

Within randomly specialising groups, each individual becomes a helper with probability p_{rand} and otherwise a reproductive. As a consequence, the actual proportion of helpers, p,

will vary from group to group. More specifically, the realised number of helpers in a group, $X(= pN)$, is a binomial random variable over N trials and with probability of success p_{rand}. In the appendix, we show that the expected fitness of the randomly specialising group is averaged over all possible group compositions ($X \in \{0,1, \dots, N\}$) giving the following expression:

$$W_{rand}(p_{rand}) \ = W_0(p_{rand})(1 - c_{rand}). \qquad (2)$$

We find that the expected fitness of a randomly specialising group is equal to the fitness of a group that has the expected proportion of helpers, $W_0(p_{rand})$, diminished by a fitness cost due to random effects, c_{rand}. That is, when the cost due to random effects increases (higher c_{rand}) the fitness of the group decreases (lower $W_{rand}(p_{rand})$). In the appendix, we show that the random cost is given by the following expression:

$$c_{rand} = \frac{(e/N) * var(X)}{W_0(p_{rand})} \qquad (3)$$

We define the numerator as the 'variance in cooperation' as it is composed of the variance in number of helpers across groups ($var(X) = pN(1 - p)$), weighted by the amount that each helper contributes to the fecundity of reproductives (e/N). The denominator is the fitness of an average group, with the expected proportion of helpers, $W_0(p_{rand})$.

We find that variance in cooperation and the fitness of an average group have opposite effects on the cost of random specialisation, c_{rand}. As the variance in cooperation across groups increases (higher $(e/N) * Var(X)$), the cost due to random effects goes up (higher

c_{rand}; see appendix). In contrast, when the fitness of a group with the expected proportion of helpers is particularly high (higher $W_0(p_{rand})$), then we find that the relative impact of variation in cooperation across groups ($(e/N) * Var(X)$) is diminished (lower c_{rand}).

Coordinated division of labour using environmental information

Groups that specialise through the use of environmental information pay no cost due to random effects ($c_{rand} = 0$), but instead always achieve a precise proportion of helpers, $p = p_{env}$, by paying a fitness scaling cost c_{env}. This cost may arise due to the metabolic expenditure required to sense and use signals or cues in the social environment (Maynard Smith and Harper, 2003). As a consequence, the fitness of this group is given by

$$W_{env}(p_{env}) = W_0(p_{env})(1 - c_{env}). \qquad (4)$$

In order to determine the conditions that favour one mechanism over the other, we must evaluate when $W_{env}(p_{env}) > W_{rand}(p_{rand})$. However, this requires specifying how p_{env} and p_{rand} are related to each other or how they are each determined. In this analysis, we presume that they are fixed and equal to one another ($p = p_{env} = p_{rand}$) as this minimizes differences between the groups and ensures that we are directly comparing the consequences of employing one mechanism over the other. For ease of simplicity we treat p as a continuously varying trait, despite the fact that p_{env} is by necessity discrete.

We proceed first by allowing the joint p to be a fixed, independent parameter of the model. This allows us to generate predictions that disentangle the separate effects of each of the

model parameters without considering how they may also impact the amount of cooperation within groups. While this can help to determine causal relationships, it is arguably unrealistic to presume such independence. We next remove this independence and instead set p equal to the optimal helper probability of a randomly specialising group ($p = p^*_{rand}$). Thus, we are allowing the average amount of cooperation to evolve and assuming that it does so to the value of p that maximises fitness when dividing labour randomly. This makes sense if we consider random specialisation to be the ancestral strategy and are evaluating when specialisation with the use of environmental information may invade such a population at equilibrium.

Which is the optimal mechanism?

We first assume that the proportion of helpers in the environmental information group and the probability of being a helper in the random group are equal ($p = p_{env} = p_{rand}$) and that this shared p is an independent parameter. As such, division by environmental information is favoured over random specialisation if $W_{env}(p) > W_{rand}(p)$. If we substitute Equations 2 and 3 into this expression, we find that division by environmental information is favoured so long as the metabolic cost of using environmental information is less than the fitness scaling cost due to random effects ($c_{env} < c_{rand}$). This expands to:

$$c_{env} < \frac{(e/N)*var(X)}{W_0(p)} \left(= \frac{ep}{N((1-e)+ep)} \right) \qquad (5)$$

This shows that division of labour by environmental information is more likely to evolve when the information cost is low (low c_{env}), when there is high cooperation within groups (high p), when cooperation is very essential for reproduction (high e) and for smaller group sizes (low N; **Figure 2**). Further, if there is no cost to using information ($c_{env} = 0$),

then we have that the above condition is trivially true and thus that division by environmental information is intrinsically the better strategy- if all else is equal, random effects are always disadvantageous.

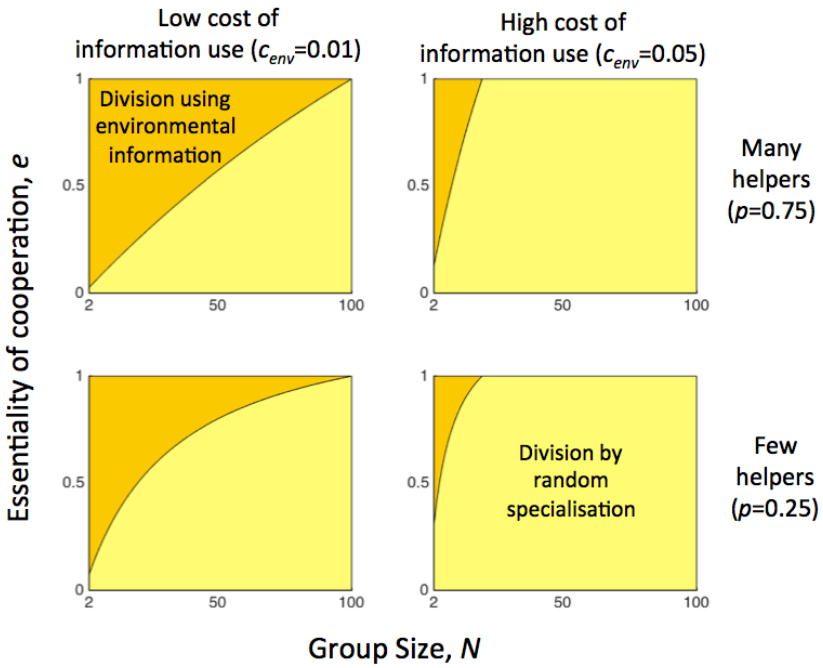


Figure 2. The optimal mechanism when the number of helpers varies independently. We find that the best way to divide labour is an adaptation that may evolve and vary between species for predictable reasons. For example, division by environmental information is more likely in small groups (low N), when cooperation is essential (high e), when there are low costs to information use (low c_{env}) and when there are many helpers (high p). In contrast, division by random specialisation is favoured in larger groups (large N), when cooperation is more of a luxury (low e) and when there are fewer helpers (low p). Here we have assumed that the proportion of helpers in a coordinated group and the helper probability in a randomly dividing group are equal ($p = p_{rand} = p_{env}$) and that the shared quantity, p, may vary independently of other model factors.

Why do these patterns emerge? That increasing costs of information use (higher c_{env}) disfavours coordinated division is unsurprising. In turn, the effects of group size (N), the essentiality of cooperation (e) and the amount of cooperation (p) all act through the cost of random specialisation, c_{rand}. For example, an increase in the essentiality of cooperation (higher e) both increases the variance in cooperation (higher $(e/N) * var(X)$) and decreases the fitness of an average group (lower $W_0(p)$) and both of these effects increase

the cost of random specialisation (higher c_{rand}). In contrast, a group size increase (higher *N*) only increases the fitness of an average group (higher $W_0(p)$), which diminishes the relative costs of random specialisation (lower c_{rand}). The amount of cooperation both disfavours and favours random specialisation via opposite effects on the variance in cooperation ($(e/N) * var(X)$) and on the fitness of an average group ($W_0(p)$), the consequence of which is always that more cooperation (higher *p*) leads to an increase in the cost of random specialisation (higher c_{rand}).

Allowing the proportion of helpers to evolve

We now consider the consequence of evaluating the relative fitness of each group when their shared *p* is set equal to the optimal helper probability of the randomly specialising group ($p = p^*_{rand}$). This corresponds to a scenario where random specialisation is the ancestral state and we wish to determine the conditions for when division by environmental information can evolve to replace it. In the appendix, we solve for p^*_{rand} and show that larger groups (high *N*) and increasing trait essentiality (high *e*) both lead to an increase in the optimal probability of being a helper (higher p^*_{rand}; **Figure 3a**). One consequence of an evolving helper probability is that division of labour is sometimes not favoured ($p^*_{rand} = 0$), particularly when the essentiality of cooperation is low (lower e). We proceed with our comparison by only considering the cases where division of labour is favoured.

In the appendix, we find that the optimal helper probability of a randomly specialising group is always less than or equal to the optimal proportion of helpers ($p^*_{rand} \leq p^*$). In particular, this difference is greater when the helping trait is more luxurious (lower *e*). When the trait is less essential (lower *e*), groups with more helpers that the optimal

proportion ($p > p^*$) are much less fit than groups with symmetrically fewer helpers than the optimal proportion ($p < p^*$). Consequently, the optimal helper probability in randomly specialised groups (p^*_{rand}) is favoured to undershoot the optimal helper proportion (p^*) in order to hedge for this asymmetry. This pattern is similar to the 'fertility insurance' that occurs in inbreeding groups with biased female-sex ratios in which the favoured stochastic proportion of males is sometimes greater than the optimal proportion in order to avoid the risk of forming groups with no males at all (West *et al.*, 2002).

By evaluating condition 4 for $p = p^*_{rand}$, we find the following condition for the evolution of division by environmental information:

$$c_{env} < \frac{e(N-1)-N(1-e)}{e(N-1)-(1-e)} \cdot \frac{1}{N}. \qquad (6)$$

We see once again that division using environmental information is broadly favoured by low cost of information use (lower c_{env}), high trait essentiality (higher e) and for small group sizes (lower N; **Figure 3c & d)**. We also find once more that division using environmental information is always favoured if there are no costs to information use ($c_{env} = 0$) (**Figure 3b**). These patterns are all in agreement with those observed for independent proportion of helpers ($p = p_{rand} = p_{env}$; **Figure 2**). However, one key exception is observed. We find that in some cases, a marginal group size increase from 2 to 3 or 4 individuals (higher N) does favour division of labour by environmental information over that by random specialising, but otherwise disfavours it in all other cases. Why do these opposing patterns emerge?

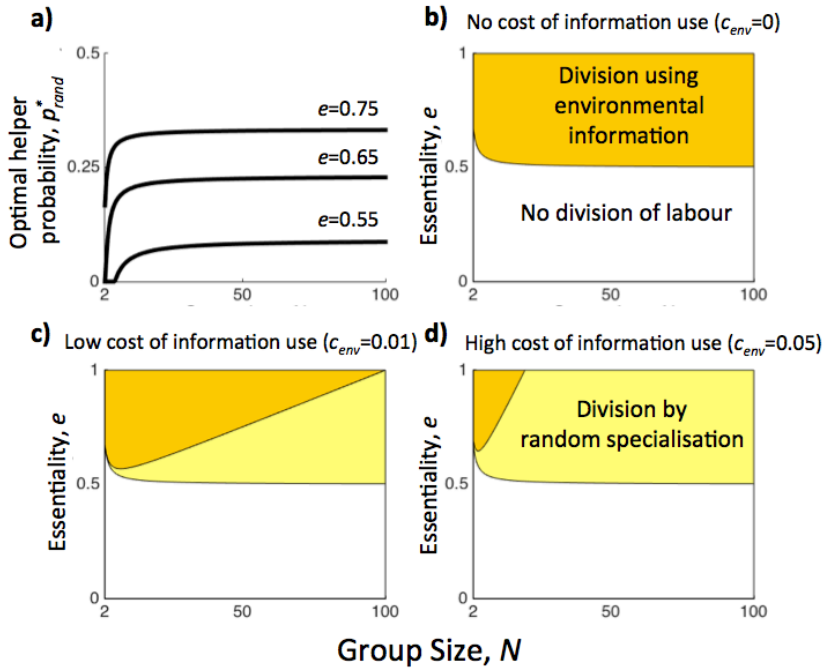


Figure 3. The optimal mechanism when the number of helpers may evolve. a) Larger groups and more essential cooperation (high N and high e) lead to randomly dividing groups with a higher optimal helper probability (higher p_1^*). If we evaluate the relative fitness of each mechanism when their shared measure of helper number (p= $p_{rand} = p_{env}$) is equal the randomly dividing optimum ($p = p^*_{rand}$), then we find the same broad results as for an independently varying p (Figure 2). b) We note that division by environmental information is always the preferred strategy if there are no costs to information use (c_{env}=0). c) & d) Otherwise, division by environmental information is favoured over random specialisation when there are costs to information use (low c_{env}), cooperation is more essential (high e) and for small group sizes (low N).

In our analysis of an independently varying p, we found that large group sizes and few helpers (high N and low p) favour random specialising (Equation 4; **Figure 2**). However, we have found here that a group size increase (high N) also leads to an increase in the optimal helper probability *(*high p^*_{rand}; **Figure 3a**). As such increasing group size (higher N) may lead to opposing effects on the likelihood that division by environmental information evolves. It just so happens that the effect of increasing group size (higher N) is predominantly to favour random specialisation through its direct effect on the random cost, c_{rand}, rather than by its effect on the helper probability, p^*_{rand}.

In the appendix, we extend this work in several ways to check that the results of our analysis are not sensitive to the assumptions of the model. First, we consider the separate cases where (a) the benefits due to the proportion of helpers may be non-linear and (b) the benefit due to helpers depends on the absolute number of helpers in the group rather than on their proportion. We also consider the more realistic scenario where the proportion of helpers in the environmental infrromation group is discrete rather than continuous. Finally we consider the possibility that the proportion of helpers in the environmental information group is set to its optimum, such that both strategies are separately optimised ($p_{rand} = p^*_{rand};\ p_{env} = p^*_{env}$). In all cases, we find the same broad patterns as in the above analysis.

Conclusion

Overall, we have found that the means by which individuals specialise when dividing labour is itself a strategy that may vary between species for adaptive reasons. Coordinated division using environmental information is more likely to evolve in smaller social groups and where the benefits of cooperation are crucial to survival and reproduction (low N and high e). In contrast, random division of labour is more likely in larger groups and where cooperation is less essential and more of a luxurious activity (high N and low e). In addition, division of labour using environmental information is more likely to evolve if the cost of information use is low (low c_{env}) and always evolves if these are negligible ($c_{env} = 0$).

Within the notable cases of division of labour in microbes, the use of environmental information or random specialisation does seem to match the broad pattern of our predictions. For example, *Salmonella enterica* and *Bacillus subtilis* populations are both comprised of large social groups (higher *N*) and where helpers are not fully essential

($e < 1$). In accordance with our model, both species divide labour by random specialisation (Ackermann *et al.*, 2008; Veening *et al.*, 2008; Veening, Smits and Kuipers, 2008). In contrast, the benefits of nitrogen fixation in cyanobacteria filaments are essential ($e = 1$) and diffuse away rapidly (lower *N*). As predicted, division of labour here is mediated by environmental cues (Flores and Herrero, 2010). At a larger scale, the everyday metabolic costs of being an animal are so much bigger than any potential cost of information use, c_{env}, that the later may be considered to be negligible for these species ($c_{env} \approx 0$). In agreement with our findings, there are hardly any instances of division of labour by random specialisation in animal societies, with the possible exception of a small non-genetic contribution to caste determination in Meliponine bees (Ratnieks, 2001).

More broadly, we may begin to ask questions about the other mechanisms for dividing labour. In particular, why is there a genetic component to specialisation in some animal societies (Smith *et al.*, 2008; Schwander *et al.*, 2010)? What are the trade-offs to such a strategy (Wahl, 2002)? On one hand, genetic differences are an easy way to achieve phenotypic differences between individuals. This limits any metabolic costs to division because no regulatory pathways are needed for differential gene expression. However, there may also be significant costs to this mechanism (Wahl, 2002; Schwander *et al.*, 2010). First, genetic differences may lead to a similar cost as that of random specialisation in that groups may form that deviate from the optimal proportion of the different genotypes (Wahl, 2002). Second, because groups cannot be clonal, there is scope for cooperation to erode as genotypes that do not perform any of the cooperative behaviours may invade (cheaters). This then would require alternate mechanisms such as sanctions and policing to maintain cooperative activities, which itself may incur a higher metabolic cost than is gained by removing regulatory pathways. A distinct possibility remains that

genetic component to specialisation are in fact not a mechanism for reproductive division of labour but rather a consequence of conflict arising after the evolution of division of labour. A general model of genetic division of labour accounting for both the metabolic trade-offs and its evolutionary stability would help in elucidating whether genetic differences are indeed a viable mechanism for such cooperation.

Appendix 1: random specialisation

In the following, we derive the expected fitness of a randomly specialising group. The expectation is taken as an arithmetic average over a binomial distribution with number of trials, N, and probability of success, p_{rand}. We denote X as the realised number of helpers within each group.

$$\begin{aligned} W_{rand}(p_{rand}) &= \sum_{X=0}^{N}(N-X)((1-e)+eX/N)\binom{N}{X}p_{rand}^{X}(1-p_{rand})^{N-X} \\ &= (1-e)(1-p)N+\left(\frac{e}{N}\right)E[X(N-X)] \\ &= (1-e)(1-p)N+ep(1-p)N-\left(\frac{e}{N}\right)Cov(X,(N-X)) \\ &= W_0(p_{rand})-(e/N)Var(X) \\ &= W_0(p_{rand})(1-c_{rand}), \end{aligned} \quad \text{(A1)}$$

where $c_{rand}=\frac{(e/N)Var(X)}{W_o(p_{rand})}$ is the relative fitness cost of random specialisation.

Appendix 2: optimal helper probability

The following is an expanded fitness equation for randomly specialising groups:

$$W_{rand}(p_{rand}) = (1-e)(1-p_{rand})N+ep_{rand}(1-p_{rand})(N-1). \quad \text{(A2)}$$

We solve here for the optimal helper probability of randomly specialising groups, p^*_{rand}. That is, the value of p^*_{rand} that maximizes Equation A2. We find that the value of p^*_{rand} that satisfies $\frac{\partial W_{rand}}{\partial p_{rand}} = 0$ is given by

$$p^*_{rand} = \frac{e(N-1)-(1-e)N}{2e(N-1)} = \frac{(2e-1)N-e}{2e(N-1)}. \quad \text{(A3)}$$

We further find that $\frac{\partial^2 W_{rand}}{\partial p^2_{rand}}\Big|_{p_{rand}=p^*_{rand}} = -2e(N-1) < 0$ and therefore the value p^*_{rand} is a maximum of the fitness landscape. Equation A3 is an increasing function of N and e.

Appendix 3: optimal proportion of helpers

The following is the fitness equation for groups that divide labour by environmental information:

$$W_{env}(p_{env}) = (1-c_{env})((1-e)(1-p_{env})N + ep_{env}(1-p_{env})N). \quad \text{(A4)}$$

We solve here for the optimal proportion of helpers, p^*_{env}. That is, the value of p_{env} that maximizes Equation A4. We find that the value of p_{env} that satisfies $\frac{\partial W_{env}}{\partial p_{env}} = 0$ is given by

$$p^*_{env} = \frac{2e-1}{2e}. \quad \text{(A5)}$$

We further find that that $\left.\frac{\partial^2 W_{env}}{\partial p_{env}^2}\right|_{p_{env}=p_{env}^*} = -2(1-c_{env})eN < 0$ and therefore the value p_{env}^* is a maximum of the fitness landscape. Equation A5 is an increasing function of e.

Appendix 4: non-linear benefits to the proportion of helpers

In our analysis, we have assumed that the fitness of reproductive increases linearly with the proportion of helpers in the group. Here we allow for non-linear dependence on the proportion of helpers by the inclusion of the parameter α. The following are the fitness equations for each mechanism in this case:

$$W_{env}(p_{env}) = (1-c_{sig})((1-e)(1-p_{env})N + e(p_{env})^{\alpha}(1-p_{env})N) \quad \text{(A6)}$$

$$W_{rand}(p_{rand}) = \sum_{X=0}^{N}(N-X)((1-e)+e(X/N)^{\alpha})\binom{N}{X}p_{rand}^{X}(1-p_{rand})^{N-X} \quad \text{(A7)}$$

An analytical solution is more difficult to come by and so we solve numerically for in order to evaluate the conditions in which $W_{env}(p_{rand}^*) > W_{rand}(p_{rand}^*)$. The results are presented in **Figure A1** and show broad agreement with the results of the linear analysis.

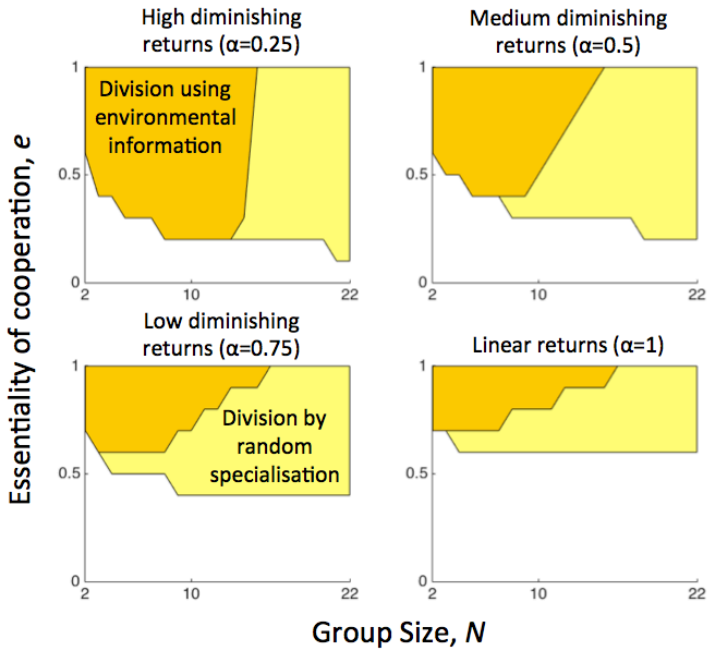


Figure A1: non-linear returns to helping. We find the same broad patterns when the fitness of reproductives depends non-linearly on the proportion of helpers as when the dependence is linear. In particular, division by environmental information is favoured when cooperation is essential (high e) and when group sizes are small (low N). The presented results are for intermediate signalling costs ($c_{env} = 0.05$). We also find broadly that division by environmental information is more likely the more that the returns are diminishing (low α).

Appendix 5: reproductives benefit from total help

In our analysis, we have assumed that the fitness of reproductive depends on the proportion of helpers in the group. Here we assume instead that the fitness of a reproductive depends on the total number of helpers in the group. The following are the fitness equations for each mechanism in this case:

$$W_{env}(p_{env}) = (1 - c_{env})((1 - e)(1 - p_{env})N + ep_{env}(1 - p_{env})N) \quad \text{(A8)}$$

$$= (1 - c_{env})W_0(p_{rand}) \quad \text{(A9)}$$

$$W_{rand}(p_{rand}) = \sum_{X=0}^{N} (N - X)((1 - e) + eX/N)\binom{N}{X} p_{rand}^{X}(1 - p_{rand})^{N-X} \quad \text{(A10)}$$

$$= (1 - c_{rand})W_0(p_{rand}) \quad \text{(A11)}$$

where we now have that $W_0(p) = (1 - \mathrm{e})(1 - \mathrm{p})\mathrm{N} + \mathrm{epN}(1 - \mathrm{p})\mathrm{N})$ and $c_{rand} = \frac{eVar(X)}{W_0(p_{rand})}$. Division by environmental information is then favoured over random specialisation at a fixed amount of cooperation, p, if $W_{env}(p) > W_{rand}(p)$, giving:

$$c_{env} < \frac{eVar(X)}{W_0(p)} = \frac{ep}{(1 - e) + epN} \quad \text{(A12)}$$

Solving for the optimum helper probability gives $p^*_{rand} = (eN - 1)/(2e(N - 1)$, which we may substitute into Equation A7 to give the condition when the amount of helping is set by the equilibrium value for the randomly specialising population. We plot the results of this analysis in **Figure A2** and find the same broad patterns of the previous analysis.

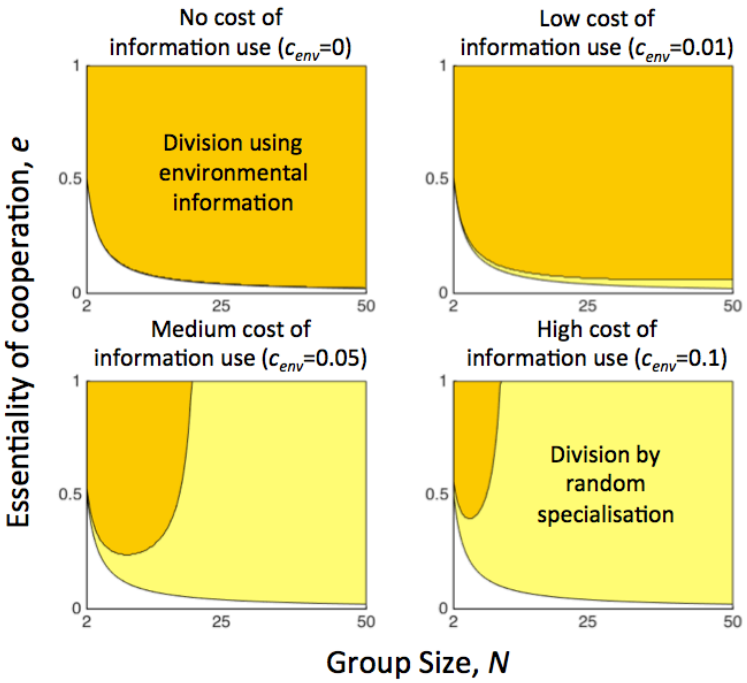


Figure A2: benefits arise from total help. We find the same broad patterns when the fitness of reproductives depends on the total number of helpers rather than on their proportion in the group.

In particular, division by environmental information is favoured when cooperation is essential (high e), broadly when group sizes are small (low N) and when signalling costs are low (low c_{sig}).

Appendix 6: discrete proportion of helpers

For ease of simplicity, we have assumed in our analysis that the proportion of helpers in the environmental information groups is a continuous character $p_{env} \in [0,1]$. In reality this quantity should be discrete as there can only be a whole number of helpers in the group. Here, we repeat our analysis, while restricting the possible values of p_{env} such that $p_{env} \in \{\frac{0,1}{N}, \frac{2}{N}, \ldots, 1\}$. This is done numerically and results are shown in supplementary **Figure A3**. We find that local patterns are more jagged but that more broadly the same results hold as in the continuous treatment.

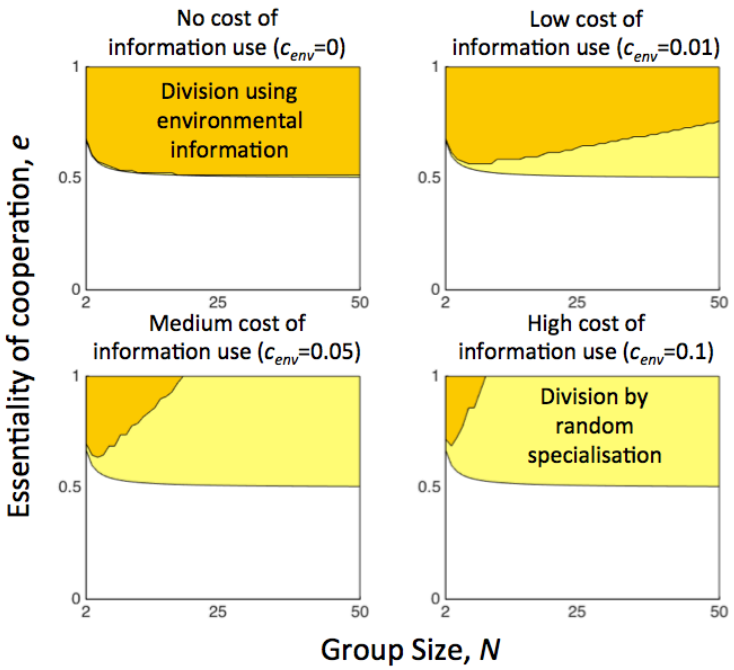


Figure A3: discrete proportion of helpers. We find the same broad patterns when the proportion of helpers in the group is forced to be dicrete. In particular, division by environmental information is favoured when cooperation is essential (high e), broadly when group sizes are small (low N) and when signalling costs are low (low c_{sig}).

Appendix 7: both mechanisms are optimised

In order to determine which is the preferred mechanism when both are optimised, we must determine which strategy has the highest average fitness $W_{env}(p^*_{env}) > W_{rand}(p^*_{rand})$. This amounts to calculating the following:

$$W_0(p^*_{env})(1 - c_{env}) \quad > \quad W_0(p^*_{rand})(1 - c_{rand}) \tag{A13}$$

where $c_{rand} = \left(\frac{e}{N}\right) p^*_{rand}\left((1 - e) + e p^*_{rand}\right)$. We solve this condition numerically and plot results in **Figure A4**. We find that the broad patterns of our analysis are retained.

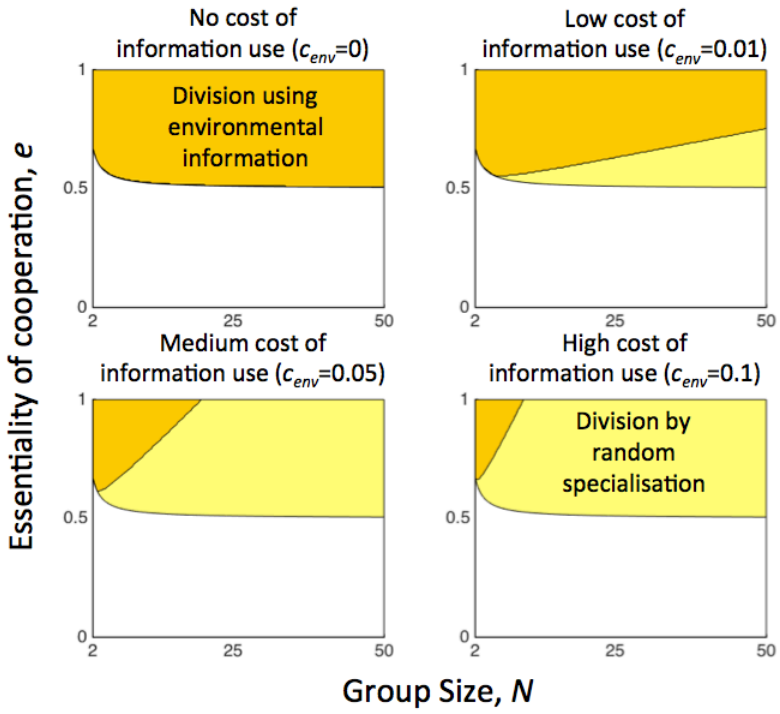


Figure A4: both mechanisms optimised. We find the same broad patterns when the proportion of helpers in the environmental information groups and the probability of being a helper in the randomly specialising groups are both optimised. In particular, division by environmental information is favoured when cooperation is essential (high e), broadly when group sizes are small (low N) and when signalling costs are low (low c_{sig}).

References

Ackermann, M. *et al.* (2008) 'Self-destructive cooperation mediated by phenotypic noise', *Nature*, 454, pp. 987–990. doi: 10.1038/nature07067.

Ackermann, M. (2015) 'A functional perspective on phenotypic heterogeneity in microorganisms', *Nature Reviews Microbiology*. Nature Publishing Group, 13, pp. 497–508. doi: 10.1038/nrmicro3491.

Boomsma, J. J. (2009) 'Lifetime monogamy and the evolution of eusociality', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, pp. 3191–3207. doi: 10.1098/rstb.2009.0101.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Cooper, G. A. and West, S. A. (2018) 'Division of labour and the evolution of extreme specialization', *Nature Ecology and Evolution*. Springer US, 2, pp. 1161–1167. doi: 10.1038/s41559-018-0564-9.

Davidson, C. J. and Surette, M. G. (2008) 'Individuality in Bacteria', *Annual Review of Genetics*, 42, pp. 253–268. doi: 10.1146/annurev.genet.42.110807.091601.

Flores, E. and Herrero, A. (2010) 'Compartmentalized function through cell differentiation in filamentous cyanobacteria', *Nature Reviews Microbiology*, 8, pp. 39–50. doi: 10.1038/nrmicro2242.

Gavrilets, S. (2010) 'Rapid transition towards the division of labor via evolution of developmental plasticity', *PLoS Computational Biology*, 6, p. e1000805. doi: 10.1371/journal.pcbi.1000805.

Ispolatov, I., Ackermann, M. and Doebeli, M. (2012) 'Division of labour and the evolution of multicellularity', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 1768–1776. doi: 10.1098/rspb.2011.1999.

Maynard Smith, J. and Harper, D. (2003) *Animal signals*. Oxford: Oxford University Press.

Michod, R. E. (2006) 'The group covariance effect and fitness trade-offs during evolutionary transitions in individuality', *Proceedings of the National Academy of Sciences*, 103, pp. 9113–9117. doi: 10.1073/pnas.0601080103.

Oster, G. F. and Wilson, E. O. (1978) *Caste and ecology in the social insects*, *Caste and ecology in the social insects*. Princeton: Princeton University Press.

Ratnieks, F. L. W. (2001) 'Heirs and spares : caste conflict and excess queen production in Melipona bees', *Behavioural Ecology and Sociobiology*, 50, pp. 467–473. doi: 10.1007/s002650100388.

Rossetti, V. *et al.* (2010) 'The evolutionary path to terminal differentiation and division of labor in cyanobacteria', *Journal of Theoretical Biology*, 262, pp. 23–24. doi: 10.1016/j.jtbi.2009.09.009.

Schwander, T. *et al.* (2010) 'Nature versus nurture in social insect caste differentiation', *Trends in Ecology and Evolution*, 25, pp. 275–282. doi: 10.1016/j.tree.2009.12.001.

Smith, C. R. *et al.* (2008) 'Genetic and genomic analyses of the division of labour in insect societies', *Nature Reviews Genetics*, 9, pp. 735–748. doi: 10.1038/nrg2429.

Veening, J.-W. *et al.* (2008) 'Transient heterogeneity in extracellular protease production by Bacillus subtilis', *Molecular Systems Biology*, 4, p. 184. doi: 10.1038/msb.2008.18.

Veening, J.-W., Smits, W. K. and Kuipers, O. P. (2008) 'Bistability, Epigenetics, and Bet-Hedging in Bacteria', *Annual Review of Microbiology*, 62, pp. 193–210. doi: 10.1146/annurev.micro.62.081307.163002.

Wahl, L. M. (2002) 'The Division of Labor: Genotypic versus Phenotypic Specialization',

The American Naturalist, 160, pp. 135–145. doi: 10.1086/340601.
West, S. A. *et al.* (2002) 'Fertility Insurance and the Sex Ratios of Malaria and Related Hemospororin Blood Parasites', *Allen Press*, 88, pp. 258–263.
West, S. A. and Cooper, G. A. (2016) 'Division of labour in microorganisms: An evolutionary perspective', *Nature Reviews Microbiology*, 14, pp. 716–723. doi: 10.1038/nrmicro.2016.111.

Chapter 5: Modelling relatedness and demography in social evolution[*]

Abstract

With any theoretical model, the modeler must decide what kinds of detail to include and which simplifying assumptions to make. It could be assumed that models that include more detail are better, or more correct. However, no model is a perfect description of reality and the relative advantage of different levels of detail depends on the model's empirical purpose. We consider the specific case of how relatedness is modeled in the field of social evolution. Different types of model either leave relatedness as an independent parameter (open models), or include detail for how demography and life cycle determine relatedness (closed models). We exploit the social evolution literature, especially work on the evolution of cooperation, to analyse how useful these different approaches have been in explaining the natural world. We find that each approach has been successful in different areas of research, and that more demographic detail is not always the most empirically useful strategy.

Introduction

Theoretical models are often used to help explain how organisms behave in the natural world (Westneat and Fox, 2010; Davies, Krebs and West, 2012). In the field of social evolution, we use theoretical models to make predictions about and to ultimately understand behaviours that affect the fitness of individuals other than the actor (Hamilton, 1964; Frank, 1998; Bourke, 2011). For example, we use models to predict when it is advantageous for individuals to cooperate; we use models to uncover the factors that

[*] Published as Cooper, G. A., Levin, S. R, Wild, G. and West, S. A. (2018) Modelling relatedness and demography in social evolution. *Evolution Letters*. 2(4), 260–271 (appended).

contribute to the origin of selfish, altruistic, and even spiteful behaviours; and we use models to account for variation in the tendency to help both within and between species.

Perhaps the most influential model in social evolution was proposed by Hamilton (1964) and showed that genetic relatedness can be a key factor in explaining the adaptive value of social behaviours. Genetic relatedness is the probability that a social partner shares the same gene at a given locus relative to that of a random individual sampled from the population (Hamilton, 1964, 1970; Grafen, 1985). In large outbreeding populations, full siblings are related by ½, half-sibs by ¼, and so on (Grafen, 1985). Individuals are favoured to help relatives as this provides an indirect opportunity to further spread identical copies of their genes into the next generation. Over the last 50 years, relatedness has proven to be a fundamental concept for explaining social behaviour across the tree of life, and theoretical models employing genetic relatedness have formed a cornerstone of social evolution (Frank, 1998; Rousset, 2004; West, 2009; Bourke, 2011)

The way in which relatedness is captured in theoretical models can be divided into two approaches, termed `open' and `closed' models (Box 1) (Taylor and Frank, 1996; Frank, 1998; Rousset, 2004; Gardner and West, 2006; Lion, Jansen and Day, 2011). In an open model, relatedness is left as an independent parameter that can be directly tuned by the theoretician without affecting the other features of the model. In a closed model, the modeler goes an extra step, to make specific assumptions about how population structure and life cycle determine relatedness. For example, the modeler might specify how model parameters, such as dispersal from the natal patch, the extent to which generations overlap, or the degree of monogamous mating impact relatedness from one generation to the next.

A potential problem with open models is that relatedness is not necessarily an independent variable (Taylor, 1992a, 1992b). The factors that determine relatedness can influence other important factors. For example, patterns of dispersal and whether generations overlap can affect both relatedness and the relative marginal costs and benefits of social traits. Consequently, assuming that relatedness is an independent parameter in an open model could give misleading predictions. In contrast, closed models can take account of how different parameters are correlated, and so could be argued to be more correct or internally consistent. Closed modelling has become the most common approach in the field of social evolution, and has been suggested as the preferable method (Lehmann and Rousset, 2010; Lion, Jansen and Day, 2011). This raises the question of whether open models should be used.

Our aim is to critically analyse the utility of both open and closed approaches. Our starting point is two propositions, which we presume are widely agreed upon: (1) All models are wrong, in that they are not an exact representation of the natural world. (2) The usefulness of any model is determined by its ability to help explain the natural world. These two points are trivially true, but there has been little guidance in the literature for empirically-minded theoreticians on when to develop one type of model over the other. We first examine the theoretical trade-offs of each approach and consider how they may be appropriate for different empirical questions. We then consider a few areas where open and closed models have been developed, including cooperation, sex allocation and dispersal. We evaluate the success of each approach in explaining empirical patterns in these areas, to see if any lessons can be drawn for future research.

The trade-offs of open and closed models

Open and closed modelling approaches differ in how they treat relatedness. Across nature, there is a wide diversity of life cycles and demographic structures that can generate relatedness between interacting individuals (Hamilton, 1964; Frank, 1998; Rousset, 2004). Some well-characterized examples include:

1. Kin discrimination – if individuals can somehow distinguish relatives from non-relatives and preferentially direct cooperation towards them, then this can generate positive relatedness between actor and recipient (Sharp *et al.*, 2005; Mehdiabadi *et al.*, 2006).
2. Dispersal patterns – limited dispersal, or dispersing as groups of relatives, can keep relatives together and hence generate positive relatedness between interacting individuals, in the absence of any kin discrimination (Hamilton, 1964).
3. Mating patterns – monogamy or lower levels of polyandry can increase the relatedness between interacting siblings (Boomsma, 2007; Hughes *et al.*, 2008; Cornwallis *et al.*, 2010; Dieter Lukas and Clutton-Brock, 2012; Cornwallis *et al.*, 2017).

Open models

An open model is agnostic about which of the above factors (or others) are responsible for the generation of relatedness between individuals. Instead, relatedness is deliberately left as an independent factor that can be tuned directly by the modeler. The benefit of this approach is that it can generate predictions that should hold across many systems, regardless of which specific demographic processes are responsible for relatedness between interacting individuals. Thus, if the model predicts that investment in a public

good will increase for higher relatedness, then this should hold just as well in systems that employ kin discrimination, limited dispersal or monogamous mating in the generation of relatedness.

The downside of an open approach is that relatedness isn't necessarily independent of other factors. For example, relatedness can be an important driver of the evolution of dispersal, but relatedness also crucially depends upon dispersal (Taylor, 1988; Frank, 1998). Open models miss such feedbacks (West *et al.*, 2002; Lehmann and Rousset, 2010). Consequently, open models may gain widespread applicability, but at a cost of demographic precision.

Closed models

In contrast, a closed model specifies the precise way in which population dynamical processes generate genetic relatedness (Table 2). In doing so, concrete assumptions must be made about the exact life cycle and demography of the system and how these factors contribute to the relatedness of interacting individuals.

The benefit of a closed-model approach is that it allows a specific question to be answered about a characterised system, in which the processes that generate relatedness are known. Any feedback effects between parameters or traits of the model with the underlying genotypic assortment in the population are captured by the model. Furthermore, because the population-genetic assumptions about relatedness are clearer, closed models lend themselves to tweaking and altering assumptions or parameters in a way that allows us to build a family of related models, for which the inter-model relationships are apparent (Table 2).

However, the final step of closing a model involves determining how a specific demography generates relatedness, precisely. Consequently, any conclusions drawn might only be applicable to that or a limited number of scenarios. This gives a precise solution, but it might be precisely irrelevant to what occurs in the real world. In fact, the way that relatedness arises in natural systems is frequently not well understood, arising from a convoluted combination of factors and processes. As such, the additional demographic assumptions that make closed models solvable are sometimes so idealized that they may add less realism to the model than might otherwise be expected (Taylor, 1992a, 1992b; Gardner and West, 2006; Lehmann and Rousset, 2010) (Table 2). Consequently, closed models gain precise demographic detail, but at a cost of broader applicability.

Open versus closed

The differences between open and closed models can be illustrated graphically. **Figure 1** graphs the relatedness (R) between interacting individuals versus the extent to which density dependent competition is at the scale of the local patch (a) (Frank, 1998). An open model can allow both these parameters to vary independently (the entire parameter space). A closed model determines how these parameters are related for a specified demography (one line on the figure). There are many different possible demographic scenarios and corresponding closed models (different lines on the figure). We provide some examples, which illustrate how different demographic assumptions can qualitatively change whether and how R and a are linked. This figure also illustrates how an open model can be used as a 'meta-model' to examine how different closed models work and relate to each other (Frank, 1998).

While there is a rough correlation between `open and closed' and 'simple and complex', this is not always the case. In principle, closed models are nested within open models – up until the point of specifying relatedness, a closed model is open (Box 1). However, in practice, not all open models are one step away from being a closed model as the demography that determines relatedness and is required to close the model may not be specified at all (Wild, 2011). Open models may instead include other ecological factors or otherwise unlinked demographic details and thus can be arbitrarily complex. Furthermore, in closed models, the interplay between different factors can sometimes lead to simpler predictions, as some parameters drop out of the analysis (Pen and Weissing, 2000). Consequently, the difference between open and closed models may often be less of a distinction in complexity rather than a differing emphasis in the kinds of details that are included.

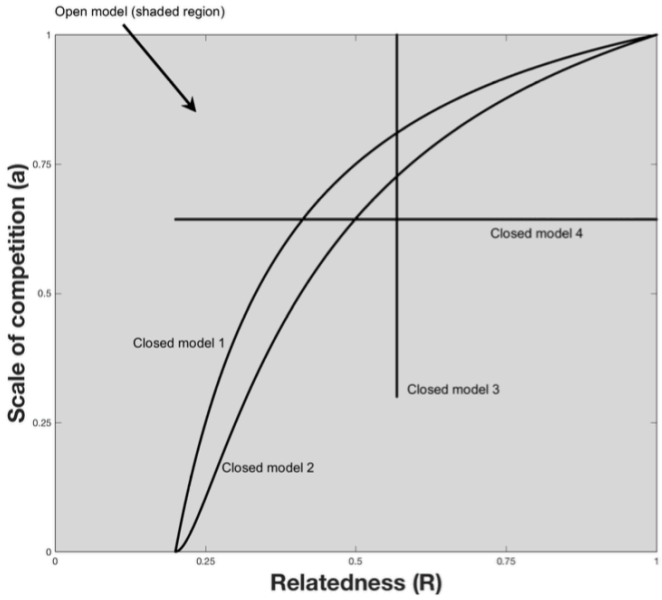


Figure 1. The relation between open and closed models. Frank (1998) developed an open model to show how local competition could reduce selection for cooperation between relatives. He used a parameter '*a*' to measure the scale at which density dependent competition occurs, which can range from completely global (a=0) to completely local (a=1). In this figure, *a* is plotted against relatedness (*R*). Frank allowed these two variables to vary independently, and so his model encompasses the entire plane (shaded grey). In a closed model, we assume a specific demography and life history, and this causes *a* and *R* to be correlated in a specific way, leading to a particular

curve in the plane (dark lines). For example, Closed model 1 is Taylor's 1992a model, closed model 2 is Taylor and Irwin's (2000) overlapping generations model, and closed models 3 and 4 are Gardner and West's 2006 budding dispersal model, for a fixed budding dispersal rate and range of migration rates, and a fixed migration rate and range of budding dispersal rates, respectively. Adapted from Gardner and West (2006).

The above is a conceptual discussion of the relative trade-offs of open and closed modelling. However, the utility of different theoretical approaches is not a philosophical question, it is something that needs to be empirically tested. What matters is the interplay between theory and data. Luckily, such an analysis is possible, via the extensive theoretical and empirical literature on the evolution of cooperation.

The evolution of cooperation: an illustrative example

A behaviour or trait is defined as cooperation if it provides a benefit to another individual, and has evolved at least partially because of this benefit (West, Griffin and Gardner, 2007b). Cooperation poses an evolutionary problem because, all else being equal, it would reduce the relative fitness of the co-operator, and hence be selected against. There is a rich theoretical and empirical literature explaining the factors that can favour cooperation (Sachs *et al.*, 2004; West, Griffin and Gardner, 2007a; Bourke, 2011).

Open models of cooperation

A potential explanation for cooperation is that it is directed towards relatives, who also carry the gene for cooperation. By helping a relative reproduce, an individual is still passing copies of its genes to the next generation, just indirectly. This process, which is usually termed kin selection, was first modeled by Hamilton (1964) (Box 1). Hamilton showed that an altruistic cooperative trait will evolve if the fitness cost to the cooperator (C) is smaller than the fitness benefit (B) to the recipient, where the benefit to the recipient is weighted by the relatedness (R) of the cooperator to the recipient: $RB - C > 0$.

This result, known as Hamilton's rule, is an open model. Relatedness is a parameter (*R*) which is treated as independent of the other parameters of the model. There is no specification of how a positive *R* arises. As such, there are a number of population- and individual-level mechanisms that could generate a given *R* value.

Hamilton's rule has been employed to explain a wide range of traits across the tree of life (Table 1). It has been used to explain behaviour, and variation in behaviour, across diverse taxa, including bacteria, slime moulds, insects, birds and mammals. The behaviours considered include many different forms of cooperation, policing, division of labour, dispersal, and harming behaviours such as killing or cannibalism. Furthermore, this includes cases where positive relatedness, or variation in relatedness, arises from a variety of factors, including limited dispersal, level of polyandry (promiscuity), kin discrimination and how groups are formed. In many cases, open models for more specific traits have also been developed (Table 1).

Closed models of cooperation

The open models discussed above black-boxed the mechanism that generated relatedness, and implicitly assumed that relatedness was independent of other model parameters. Over the last 30 years, many modelers interested in cooperation have instead employed closed models (Table 2).

Hamilton (1964) recognized that population viscosity via limited dispersal is a key mechanism for generating the positive relatedness values that can favour cooperation in Hamilton's rule. At the same time, however, limited dispersal can also increase

competition between relatives, which reduces the relative benefit of helping relatives (Hamilton, 1971, 1975). It is possible to put this local competition into an open model by adding an extra independent parameter or parameters (Grafen, 1984; Frank, 1998; Grafen and Archetti, 2008). For example, RB-C-R_2D_2, where R_2 is the average relatedness between the actor and the individuals that suffer from increased competition and D_2 is the cost to these individuals (Grafen, 1984). However, when parameters such as R and R_2 or B and D_2 are determined by the same factors, they will be correlated. Consequently, keeping them as independent parameters could give misleading predictions. For example, if limited dispersal increases both R and R_2, then we might not expect a higher relatedness (R) to lead to higher cooperation.

Taylor (1992a) developed a closed model of cooperation that considered the explicit effects of social group size and dispersal rates. He then estimated the value of relatedness as generated by the specific life history details of the model. In a landmark result, he found that the dispersal rate had no influence on the evolution of cooperation. In Taylor's model, the effect of increased relatedness and competition exactly cancel. As such, Taylor's closed model predicted that a decrease in dispersal (and therefore an increase in relatedness) would not favour cooperation as predicted by the simple form of Hamilton's rule. As well as this specific result, for that exact life history, Taylor's model makes a general point about how we need to consider both cooperation and competition between relatives.

Taylor's model has since been expanded into a number of other closed models that tweak the life history in some manner (Table 2). In many of these cases, the specific life cycle allows limited dispersal to increase relatedness (*R*), without being exactly cancelled by a

decreased benefit to relatives (*B*). Consequently, in these models, limited dispersal can favour cooperation. For example, Taylor and Irwin (2000) found that overlapping generations increase relatedness without inflating the costs of competition. This happens because there is a population-level mechanism (parent survival) for genetic associations to accrue in the absence of extra offspring remaining on the patch and competing (Box 1).

However, these closed models have had relatively little impact on our empirical understanding of specific biological cases. There is only one empirical example from the natural world where the data suggests that the influence of dispersal rates on relatedness and competition exactly cancel out – competition for mates between male fig wasps (West *et al.*, 2001). The closed models stimulated experimental evolution studies in bacteria, examining how patterns of dispersal can influence both relatedness and competition (Griffin, West and Buckling, 2004; Kümmerli *et al.*, 2009). However, these studies can be seen as 'wet simulations' that support theory, but do not measure the consequences of competition in nature. Further, the role of demographic details has been discussed but rarely tested in a number of taxa, including RNA replicators, birds, and killer whales (Hatchwell, 2009; Johnstone and Cant, 2010; Croft *et al.*, 2017; S. Levin and West, 2017).

Open versus closed

Why have open models been more useful for explaining specific empirical examples of cooperation? We suggest seven, non-mutually exclusive possibilities: (i) a closed model specifies a certain demography, narrowing the organisms to which it can be applied; (ii) closed models include an additional layer of demographic detail, which can make them more complex, and harder for empiricists to apply (or at least, they appear to); (iii) open models can offer intuitive heuristics, like Hamilton's rule, which can be applied broadly,

generate simple predictions, and facilitate interpretation of results; (iv) open models make predictions in terms of *R*, which will often be a relatively easy parameter to measure; (v) open models disentangle causal effects in similar way to experiments that try to manipulate single factors while keeping everything else fixed; (vi) open models can focus on other biological details of potential interest, rather than demography (e.g. partner sanctions, or how cooperative benefits are shared; West *et al.*, 2002; Cooper and West, 2018); and (vii) there may not be enough two-way interactions between those developing the theory and those collecting the data.

The utility of the different approaches can also be illustrated by imagining a hypothetical scenario in which theoretical work on cooperation had started with Taylor's (1992a) closed model. In this case, we would have been left with the prediction that limited dispersal (higher relatedness) does not favour cooperation. Empirically this is clearly not the case, as limited dispersal appears to play a key role in favouring cooperation in a broad range of taxa (Table 1). But, at the same time, Taylor's model has been incredibly influential in its own right. The point is that Taylor's closed model was useful when discussed *against* an open model (Hamilton's rule). Hamilton's rule said relatedness matters, and it clearly does (Table 1). Taylor's model showed that, in certain cases, things could be more complicated as competition can reduce selection or even negate selection for cooperation between relatives. This helped us explain the data from fig wasps and stimulated experiments on bacteria (West *et al.*, 2001; Griffin, West and Buckling, 2004; Kümmerli *et al.*, 2009), and led to a large body of theoretical work (Lehmann and Rousset, 2010; Van Cleve and Lehmann, 2013; Peña, Nöldeke and Lehmann, 2015; Van Cleve, 2015). Furthermore, the combination of open and closed models in this area also spurred work on how local

competition can favour spiteful harming behaviours (Gardner, West and Buckling, 2004; Lehmann, Bargum and Reuter, 2006; Gardner *et al.*, 2007).

Beyond cooperation

How useful have open and closed models been more generally? Another area of social evolution where there has been productive interplay between theory and data is the study of how organisms allocate resources to male and female offspring, termed sex allocation (West, 2009). Within this area, the two relevant success stories are: (1) local mate competition (LMC) – how population structuring, with competition for mates between related males, selects for female biased sex ratios (Hamilton, 1967); (2) sex allocation driven by relatedness asymmetries in haplodiploid social insects (Trivers and Hare, 1976; Boomsma and Grafen, 1991). Closed and open models have driven research in these two areas respectively, demonstrating that, in different fields, one approach has sometimes been more useful than the other.

Hamilton (1967) showed that if n diploid females lay eggs on a patch, if mating then occurs on this patch, and if only the females disperse to compete globally, then the evolutionarily stable strategy is to invest a fraction $(n\text{-}1)/2n$ of resources into female offspring. The beauty of this closed model is that it is an excellent approximation of the life history of many species, and leads to a prediction in terms of one parameter that is often relatively easy to measure (n). A closed model works so well here, because clear morphological features, such as non-dispersing wingless males, enforce life-history features that facilitate mathematical simplifications. Hamilton's LMC model has proved extremely useful for explaining variation in sex allocation, both within and between species (West 2009). Furthermore, theory has been extended in numerous directions to

account for life history and demographic details relevant to certain species (West, 2009). Alternative open formulations of Hamilton's LMC equation are possible, which focus on the relatedness between male and female offspring on a patch, but these can be less easy to apply (Frank, 1998; Nee, West and Read, 2002).

Boomsma & Grafen (1991) showed that, in haplodiploid social insects, workers are favoured to adjust the colony sex allocation in response to the relatedness structure within their colony. They produced an open model, and outlined how relatedness structure could be determined by a number of demographic factors, including queen mating rate, queen number, worker reproduction and queen replacement. Their model is able to explain considerable variation in sex allocation, between colonies (split sex ratios), in response to these factors (West 2009). A single open model could be applied across, and therefore unify, a number of different scenarios, where different features of the demography drive 'split sex ratios'. Together, these examples from sex allocation highlight that, for distinct empirical questions, different approaches have been more useful.

There are other areas where open or closed models have been more important for the development of theory. For example, closed models have dominated theoretical work on the evolution of dispersal, because the dispersal rate is both the trait under selection and the determinant of relatedness (Taylor, 1988; Frank, 1998; Gandon, 1999; Gandon and Michalakis, 1999; Gandon and Rousset, 1999; Rousset, 2004). Another example is the evolution of virulence, where early models tended to be open whereas later models are predominately closed (Frank, 1996b; Gandon and Michalakis, 2000; Wild, Gardner and West, 2009; Alizon and Lion, 2011; Lion, 2013). However, neither of these fields have led to a similar interplay between theory and data, possibly because most of the theory was not

developed to address specific empirical patterns (Crespi and Taylor, 1990; Innocent *et al.*, 2010).

Finally, there are also parameters other than relatedness that could be left open or closed. For example, in models where populations are structured into different classes—such as age, sex or size—reproductive values are usually treated as closed. However, open models could be developed in these cases by employing a conservation of reproductive value criterion. Because total reproductive value of the population is constant, an increase in the reproductive value of one individual necessitates exact compensatory changes in the reproductive value of others, allowing the modeller to keep this as an open parameter (e.g. Wild and West, 2007). Exactly how our argument extends to these other questions remains unclear.

Guidelines

An obvious take home is that the different approaches have different utilities. But this is a bit vague and obvious. Can a summary of our above discussion provide more specific guidelines?

Open models have proved more useful for when we want to consider cases where multiple demographic and life history details can influence relatedness. For example, how limited dispersal, kin discrimination and female mating rate influence the evolution of cooperation, or how queen mating rate, queen number, and queen replacement influence the evolution of split sex ratios (Hamilton, 1964; Boomsma and Grafen, 1991). In these cases, an open model can be applied broadly across diverse taxa, with very different life

cycles. In addition, open models have been useful for providing conceptual unification, and intuitive heuristics for guiding empirical work.

Closed models have proved particularly useful when a single demographic factor is more universally important. For example, how the number of females laying eggs per patch influences sex allocation (Hamilton, 1967). In such cases, a closed model can be applied broadly across different taxa, which share this key aspect of their life cycle. In addition, closed models have been useful conceptually for disentangling the roles of different demographic parameters.

More generally, with all these considerations, the emphasis should always be on the interplay between theory and data, and how the theory will be used to help us explain the natural world. When developing theory, there are a number of empirically motivated questions to be asked. What aspect of the empirical data can't be explained by existing theory and needs a new model? What are the parameters that empirical work suggests need more attention? Do we want to make broad predictions across species with different life cycles, or for a single species with a specific life cycle? The advantage of more empirically minded development of theory is clearly illustrated by the success of closed models developed to examine sex allocation (local mate competition), compared to those for cooperation and dispersal. In particular, the extensions of basic local mate competition theory have proven very useful precisely because their development was driven by cases where the data and/or life history assumptions did not fit existing theory (West 2009).

Conclusions

To conclude, open and closed models are complementary and not competing approaches. Ultimately, we must ask what the modeler is prepared to give up, and what they want to gain, which will depend on the modeler's empirical aim. Sylvain Gandon pointed out to us that an analogy here can be made with the analysis of statistical data. If the addition of an extra variable does not significantly improve the explanation of the data, then the more detailed model, with that extra variable, can be a less good model, as judged by statistical measures such as AIC. An important goal should be to develop a model with the minimal level of detail required to answer a specific biological question (May, 2004). Evaluating whether to use an open or closed model is then simply a matter of determining where that minimal level of detail falls with respect to demography and population structure

Finally, this debate touches on a recurring theme in behavioural and evolutionary ecology, where there are numerous examples of different potential approaches. Some examples include population genetics versus game theory, general versus specific models in game theory, or experimental studies on a specific species versus across species comparative studies (Parker and Maynard Smith, 1990; Harvey and Purvis, 1991; Davies, Krebs and West, 2012). All of these cases have generated arguments that one approach is 'better' or 'more correct' than the other whereas, in reality, the different methodologies have different strengths and weaknesses and are each appropriate in different scenarios.

Acknowledgements

We thank Sylvain Gandon, Miguel dos Santos, Andy Gardner, Michael Cant and one anonymous reviewer for their helpful comments and discussion.

Taxa	**Trait / Phenomena explained**	**Cause of variation in *R***	**Empirical approach**	**More specific open models**
Bacteria	Public goods (extracellular factors)	Dispersal pattern	Experimental evolution (Griffin, West and Buckling, 2004)	(Brown, 1999; West and Buckling, 2003; Dionisio and Gordo, 2006; Frank, 2010)
Bacteria	Quorum sensing	Dispersal pattern	Experimental evolution (Diggle *et al.*, 2007; Rumbaugh *et al.*, 2012; Pollitt *et al.*, 2014; Popat *et al.*, 2015)	(Brown and Johnstone, 2001)
Bacteria	Killing (bacteriocins)	Kin discrimination, dispersal pattern	Experimental (Inglis *et al.*, 2009)	(Gardner, West and Buckling, 2004)
Bacteria	Symbiotic benefit	Dispersal pattern (transmission)	Comparative (Fisher *et al.*, 2017)	(Frank, 1996a)
Birds & mammals	Cooperative breeding	Level of polyandry	Comparative (Cornwallis *et al.*, 2010, 2017; D. Lukas and Clutton-Brock, 2012; Dieter Lukas and Clutton-Brock, 2012)	(Charnov, 1981)
Birds & mammals	Cooperation	Kin discrimination	Observational, experimental, comparative (Komdeur, 1994; Russell and Hatchwell, 2001; Griffin and West, 2003; Komdeur, Richardson and Burke, 2004; Sharp *et al.*, 2005; Cornwallis, West and Griffin, 2009)	-
Fungus	Cooperation	Group formation, kin discrimination	Experimental evolution (Bastiaans, Debets and Aanen, 2016)	-
Insects	Eusociality	Level of polyandry	Comparative (Hughes *et al.*, 2008)	(Charnov, 1978, 1981; Gardner, Alpedrinha and West, 2012;

				Alpedrinha *et al.*, 2013; Alpedrinha, Gardner and West, 2014; Rautiala, Helanterä and Puurtinen, 2014; Liao, Rong and Queller, 2015)
Insects	Policing	Level of polyandry	Experimental, Comparative (Ratnieks, Foster and Wenseleers, 2006; Wenseleers and Francis L. W. Ratnieks, 2006; Wenseleers and Francis L.W. Ratnieks, 2006)	(Ratnieks, 1988; Wenseleers and Ratnieks, 2004; Wenseleers, Hart and Ratnieks, 2013)
Insects	Killing	Haplodiploidy, dispersal pattern, kin discrimination	Observational, experimental (Grbic, Ode and Strand, 1992; Giron *et al.*, 2004; Giron, Pincebourde and Casas, 2004)	-
Insects	Reproductive restraint	Level of polyandry	Observational, comparative (Wenseleers and Ratnieks, 2004)	(Wenseleers, Ratnieks and Billen, 2003; Wenseleers, Hart and Ratnieks, 2013)
Salamanders	Cannibalism	Kin discrimination	Experimental (Pfennig and Collins, 1993; Pfennig, Sherman and Collins, 1994; Pfennig, Collins and Ziemba, 1999)	-
Slime moulds	Fruiting bodies	Dispersal pattern, kin discrimination	Observational, experimental evolution, genomic (Mehdiabadi *et al.*, 2006; Gilbert *et al.*, 2007; Kuzdzal-Fick *et al.*, 2011; Ostrowski *et al.*, 2015; Noh *et al.*, 2018)	-
Social groups of	Division of	Dispersal pattern	Comparative	(Cooper and West, 2018)

cells (across taxa)	labour, sterile cells		(Fisher, Cornwallis and West, 2013)	

Table 1. Examples of some of the phenomena where an open model approach (Hamilton's rule) has helped us understand biological phenomena. Our list is illustrative, not exhaustive, and we provide examples of the consequences of variation in only a single parameter (*R*). More specific open models are often constructed for specific traits. In many cases, some form of Hamilton's rule emerges as a prediction and is useful for interpreting these models (Taylor and Frank, 1996; Frank, 1998). For some other traits, such as sex allocation, the results are still interpreted with kin selection, but Hamilton's rule *per se* is less useful for interpretation. Studies focusing on the consequences of variation in other parameters (*B, C*), and whether Hamilton's rule is satisfied, are reviewed elsewhere (Bourke, 2011, 2014).

Theoretical models	**Process modeled**	**When does limited dispersal favours cooperation?**
(Taylor, 1992a)	Patch elasticity	Always
(Taylor and Irwin, 2000; Irwin and Taylor, 2001; S. R. Levin and West, 2017)	Overlapping generations	When generations overlap
(Gardner and West, 2006; Lehmann, Perrin and Rousset, 2006; Traulsen and Nowak, 2006; Lehmann *et al.*, 2007)	Budding dispersal	When individuals are more likely to disperse together than singly (budding).
(Rogers, 1990)	Selective emigration	If altruists are more likely to emigrate
(Johnstone and Cant, 2008; Gardner, 2010)	Sex-specific dispersal	When the sex with higher variance in fitness is (slightly) more likely to disperse
(Johnstone, 2008; Lehmann, Ravigné and Keller, 2008)	Caste-specific dispersal	When different castes (e.g. queen and worker) have different dispersal rates, reproductive values, and dispersal timings
(Alizon and Taylor, 2008)	Empty sites	When there are empty sites on patches
(El Mouden and Gardner, 2008)	Conditional helping	When co-operators adjust their behaviour conditional on whether they disperse
(Kelly, 1992; Taylor, 1992b; Queller, 1994; Gardner and West, 2006)	Various timings of cooperation and competition	Under some but not all demographic timing schemes
(Yeh and Gardner, 2012)	Different ploidies	Under some but not all ploidies
(Rodrigues and Gardner, 2012, 2013a, 2013b)	Heterogeneity in patch quality, group size, and individual quality	When patches vary spatially and temporally in patch quality and group size, and (under some circumstances) when individuals vary in quality
(Perrin and Lehmann, 2001)	Kin discrimination	When individuals can actively discriminate kin

Table 2. Examples of the ways that Taylor's (1992a) model has been extended to incorporate additional biological details (non-exhaustive). We focus here on analytical models (rather than simulations), as these allow us to the see the explicit role of different parameters. We focus on island models, as opposed to spatially explicit models (e.g. lattice

or stepping stone), as the added mathematical complexity of these models makes it harder to interpret parameter relationships, without necessarily revealing patterns that can't already be identified in simpler island models (Lehmann and Rousset 2010). A number of other models have used different approaches (e.g. lattice models, cellular automata, evolution on graphs) to identify a number of other factors that can alleviate the effects of local competition (e.g. Van Baalen and Rand, 1998; Mitteldorf and Wilson, 2000; Ohtsuki *et al.*, 2006; Lehmann *et al.*, 2007; Lion and Gandon, 2009).

Box 1 | Open and closed: a toy model

We develop a simple model of public goods, first with an open and then a closed approach, to illustrate the two methods. We model the most general form of a public good, following Hamilton (1964), Taylor (1992a, 1992b), and Frank (2010). We take an inclusive fitness approach because the fitness derivations are simpler in this case, though an equivalent direct (neighbour modulated) fitness approach can be found in Taylor et al. (2007) and Levin and West (2017b).

Open Model: Some organism, such as a microbe, produces some costly public good, the benefits of which are shared between its social partners and itself. Examples in nature of public goods include the production and release of molecules by bacteria that scavenge for iron or digest protein (Griffin et al 2004, Diggle et al 2007). Because the production of the public good is costly to the individual, we might expect natural selection to favour individuals that don't incur the cost of production, but reap the benefits of good-producing social partners. Thus, we are interested in the conditions that would favour the evolution of the public good producing trait.

We assume an infinite population of individuals subdivided into social groups of size N (the infinite island model). Individuals can produce the public good at some private fecundity cost, c, which provides some fecundity benefit, b, to all individuals on the patch (including the focal individual). Hamilton (1964, 1970) showed that a trait will spread if its

inclusive fitness effect, W_{IF} , is greater than *0* ($W_{IF} > 0$), where the inclusive fitness effect of an actor's trait is its effect on all individuals in the population, weighted by relatedness of the actor to those affected individuals (including the actor itself), or 'recipients'. In this case, the trait has a negative cost to the actor (with relatedness 1), and the relatedness to recipients is *r*, the average whole group relatedness in a social group (as opposed to others-only relatedness). Thus the trait will spread if:

$$rb - c > 0,$$

which is a simple form of Hamilton's (1964) rule with b and c as simple additive fitness effects, as opposed to the general, regression form of Hamilton's rule (Gardner, West and Wild, 2011). This is an open model, in which the mechanism by which *r* is generated is undefined. Positive relatedness in this model could come about through limited dispersal, kin recognition, partner choice, or any other process that generates genetic correlations within social groups. However, if *r* is correlated with the other model parameters (*b* and *c*), the predictions of this model might not be very useful for explaining variation in nature.

Closed Model: We might, for example, be interested in the case in which relatedness is generated through limited dispersal. We can capture this by incorporating a new parameter, *d*, which measures the proportion of offspring that disperse from their natal social group (with a fraction *(1-d)* remaining in the group). Following Taylor (1992a), we must now take into account not only the offspring produced as a direct result of public goods production, but also those offspring indirectly displaced as a result of the cooperative trait. An individual that expresses the public good trait incurs a fecundity cost, *c*, with relatedness 1, and provides a fecundity benefit, *b*, to recipients whose average

relatedness is *r*. These extra *(b – c)* offspring remain in the social group with probability *(1 – d)*, in which case the individuals they displace are also native with probability *(1 – d*), and therefore have relatedness r. The overall inclusive fitness effect, then, is

$$W_{IF} = rb - c - r(1-d)^2(b-c)$$

The above is still an open model, assuming independence between *r* and model parameters. This illustrates that in principle, up until this point open and closed models can incorporate the same amount of demographic detail (though in practice, open models often don't). (Taylor, 1988, 1992a) showed how we can close the model by making additional assumptions, and taking an extra step of. Specifically, he calculated relatedness in terms of the demographic parameters of the model (*d* & *N*). We can do this by writing the following population genetic recursion for the change in relatedness in a social group from one generation to the next:

$$r_{t+1} = 1/N + r_t(1-d)^2(N-1)/N$$

Where the first term is the chance that two randomly sampled individuals on the patch are the same individual, and have relatedness one, and the second term is the chance they are different individuals both native to the patch, and therefore have the relatedness from the previous generation. Solving for the equilibrium value of relatedness, and plugging into the inclusive fitness effect above, we find the condition for the trait to spread is:

$$b/N > c$$

This is Taylor's classic result—that the dispersal rate has no impact on whether the trait will spread.

Extensions: we can extend this closed model a number of ways to look at the impact of different life histories and explicit demographic parameters (Table 2). We do this by rewriting the fitness function *and* recalculating our estimate of relatedness accordingly. As one example, Taylor and Irwin (2000) allowed for overlapping generations by including a parameter *s*, the probability that a parent survives into the next generation. The inclusive fitness effect becomes:

$$W_{IF} = (1 - s)[(rb - c) - r(1 - d)^2(b - c)$$

Plugging in the equilibrium relatedness value, calculated in terms of *s*, *d*, and *N*, the condition for the public good trait to evolve becomes:

$$b/c > N - (N - 1)[(2s(1-d))/((2 - d)(1 + s))]$$

The Scale of Competition

Open models can be used to provide an alternate way to look at the factors that arise in closed models (Frank 1998, Gardner & West 2006). For example, Frank (1998) developed a model for incorporating competition into an open model, by subsuming the scale of competition into benefit term of Hamilton's rule:

$$RB - C > 0$$

Where R = r, C = c, and B = b – a(b – c), and a is the proportion of competition that happens locally.

Queller (1994) developed a similar approach in which competition is subsumed into the relatedness parameter:

$$RB - C > 0$$

Where B = b, C = c, and R = (r – ar)/ (1 – ar), and therefore relatedness is not to an average member of the population but an average competitor. Both the Queller (1994) and Frank (1998) approaches recover Taylor's (1992a) result as a specific case (see Gardner and West 2006 for further discussion).

References

Alizon, S. and Lion, S. (2011) 'Within-host parasite cooperation and the evolution of virulence', *Proceedings of the Royal Society B: Biological Sciences*, 278, pp. 3738–3747. doi: 10.1098/rspb.2011.0471.

Alizon, S. and Taylor, P. (2008) 'Empty sites can promote altruistic behavior', *Evolution*, 62, pp. 1335–1344. doi: 10.1111/j.1558-5646.2008.00369.x.

Alpedrinha, J. *et al.* (2013) 'Haplodiploidy and the evolution of eusociality: worker reproduction', *The American Naturalist*, 182, pp. 421–438. Available at: http://www.journals.uchicago.edu/doi/10.1086/677283.

Alpedrinha, J., Gardner, A. and West, S. A. (2014) 'Haplodiploidy and the Evolution of Eusociality: Worker Revolution', *The American Naturalist*, 184, pp. 303–317. doi: 10.1086/677283.

Van Baalen, M. and Rand, D. A. (1998) 'The unit of selection in viscous populations and the evolution of altruism', *Journal of Theoretical Biology*, 193, pp. 631–648. doi: 10.1006/jtbi.1998.0730.

Bastiaans, E., Debets, A. J. M. and Aanen, D. K. (2016) 'Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters', *Nature Communications*, 7, pp. 1–10. doi: 10.1038/ncomms11435.

Boomsma, J. J. (2007) 'Kin Selection versus Sexual Selection: Why the Ends Do Not Meet', *Current Biology*, 17, pp. R672–R683. doi: 10.1016/j.cub.2007.06.033.

Boomsma, J. J. and Grafen, A. (1991) 'Colony-level sex ratio selection in the eusocial Hymenoptera', *Journal of Evolutionary Biology*, 4, pp. 383–407. doi: 10.1046/j.1420-9101.1991.4030383.x.

Bourke, A. F. G. (2014) 'Hamilton's rule and the causes of social evolution', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369, p. 20130362. doi: 10.1098/rstb.2013.0362.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Brown, S. P. (1999) 'Cooperation and conflict in host-manipulating parasites', *Proceedings of the Royal Society B: Biological Sciences*, 266, pp. 1899–1904. doi: 10.1098/rspb.1999.0864.

Brown, S. P. and Johnstone, R. A. (2001) 'Cooperation in the dark: Signalling and collective action in quorum-sensing bacteria', *Proceedings of the Royal Society B: Biological Sciences*, 268, pp. 961–965. doi: 10.1098/rspb.2001.1609.

Charnov, E. L. (1978) 'Evolution of eusocial behavior: Offspring choice or parental parasitism?', *Journal of Theoretical Biology*, 75, pp. 451–465. doi: 10.1016/0022-5193(78)90356-9.

Charnov, E. L. (1981) 'Kin selection and helpers at the nest: Effects of paternity and biparental care', *Animal Behaviour*, 29, pp. 631–632. doi: 10.1016/S0003-3472(81)80130-3.

Van Cleve, J. (2015) 'Social evolution and genetic interactions in the short and long term', *Theoretical Population Biology*, 103, pp. 2–26. doi: 10.1016/j.tpb.2015.05.002.

Van Cleve, J. and Lehmann, L. (2013) 'Stochastic stability and the evolution of coordination in spatially structured populations', *Theoretical Population Biology*, 89, pp. 75–87. doi: 10.1016/j.tpb.2013.08.006.

Cooper, G. A. and West, S. A. (2018) 'Division of labour and the evolution of extreme specialization', *Nature Ecology and Evolution*. Springer US, 2, pp. 1161–1167. doi: 10.1038/s41559-018-0564-9.

Cornwallis, C. K. *et al.* (2010) 'Promiscuity and the evolutionary transition to complex

societies', *Nature*, 466, pp. 969–972. doi: 10.1038/nature09335.
Cornwallis, C. K. *et al.* (2017) 'Cooperation facilitates the colonization of harsh environments', *Nature Ecology and Evolution*, 1, p. 0057. doi: 10.1038/s41559-016-0057.
Cornwallis, C. K., West, S. A. and Griffin, A. S. (2009) 'Routes to indirect fitness in cooperatively breeding vertebrates: Kin discrimination and limited dispersal', *Journal of Evolutionary Biology*, 22, pp. 2445–2457. doi: 10.1111/j.1420-9101.2009.01853.x.
Crespi, B. J. and Taylor, P. D. (1990) 'Dispersal Rates Under Variable Patch Density', *The American Naturalist*, 135, pp. 48–62. doi: 10.1086/285031.
Croft, D. P. *et al.* (2017) 'Reproductive Conflict and the Evolution of Menopause in Killer Whales', *Current Biology*, 27, pp. 298–304. doi: 10.1016/j.cub.2016.12.015.
Davies, N. B., Krebs, J. R. and West, S. A. (2012) *An Introduction to Behavioural Ecology*, *An introduction to behavioural ecology*. Hoboken, New Jersey: John Wiley & Sons. doi: 10.1037/026600.
Diggle, S. P. *et al.* (2007) 'Cooperation and conflict in quorum-sensing bacterial populations', *Nature*, 450, p. 411. doi: 10.1038/nature06279.
Dionisio, F. and Gordo, I. (2006) 'The Tragedy of the Commons, the Public Good Dilemma and the Meaning of Rivalry and Excludability in Evolutionary Biology', *Evolutionary Ecology Research*, 8, pp. 321–332. doi: 10.1016/j.apm.2009.11.005.
Fisher, R. M. *et al.* (2017) 'The evolution of host-symbiont dependence', *Nature Communications*, 8, p. 15973. doi: 10.1038/ncomms15973.
Fisher, R. M., Cornwallis, C. K. and West, S. A. (2013) 'Group formation, relatedness, and the evolution of multicellularity', *Current Biology*, 23, pp. 1120–1125. doi: 10.1016/j.cub.2013.05.004.
Frank, S. A. (1996a) 'Host-symbiont conflict over the mixing of symbiotic lineages', *Proceedings of the Royal Society B: Biological Sciences*, 263, pp. 339–344. doi: 10.1098/rspb.1996.0052.
Frank, S. A. (1996b) 'Models of Parasite Virulence', *The Quarterly Review of Biology*, 71, pp. 37–78. doi: 10.1086/419267.
Frank, S. A. (1998) *Foundations of Social Evolution*, *Monographs in behavior and ecology*. Princeton: Princeton University Press. doi: 10.1038/sj.hdy.6885351.
Frank, S. A. (2010) 'A general model of the public goods dilemma', *Journal of Evolutionary Biology*, 23, pp. 1245–1250. doi: 10.1111/j.1420-9101.2010.01986.x.
Gandon, S. (1999) 'Kin competition, the cost of inbreeding and the evolution of dispersal', *Journal of Theoretical Biology*, 200, pp. 345–364. doi: 10.1006/jtbi.1999.0994.
Gandon, S. and Michalakis, Y. (1999) 'Evolutionarily stable dispersal rate in a metapopulation with extinctions and kin competition', *Journal of Theoretical Biology*, 199, pp. 275–290. doi: 10.1006/jtbi.1999.0960.
Gandon, S. and Michalakis, Y. (2000) 'Evolution of parasite virulence against qualitative or quantitative host resistance', *Proceedings of the Royal Society B: Biological Sciences*, 267, pp. 985–990. doi: 10.1098/rspb.2000.1100.
Gandon, S. and Rousset, F. (1999) 'Evolution of stepping-stone dispersal rates', *Proceedings of the Royal Society B: Biological Sciences*, 266, pp. 2507–2513. doi: 10.1098/rspb.1999.0953.
Gardner, A. *et al.* (2007) 'Spiteful Soldiers and Sex Ratio Conflict in Polyembryonic Parasitoid Wasps', *The American Naturalist*, 169, pp. 519–533. doi: 10.1086/512107.
Gardner, A. (2010) 'Sex-biased dispersal of adults mediates the evolution of altruism among juveniles', *Journal of Theoretical Biology*, 262, pp. 339–345. doi:

10.1016/j.jtbi.2009.09.028.
Gardner, A., Alpedrinha, J. and West, S. A. (2012) 'Haplodiploidy and the Evolution of Eusociality: Split Sex Ratios', *The American Naturalist*, 179, pp. 240–256. doi: 10.1086/663683.
Gardner, A. and West, S. A. (2006) 'Demography, altruism, and the benefits of budding', *Journal of Evolutionary Biology*, 19, pp. 1707–1716. doi: 10.1111/j.1420-9101.2006.01104.x.
Gardner, A., West, S. A. and Buckling, A. (2004) 'Bacteriocins, spite and virulence', *Proceedings of the Royal Society B: Biological Sciences*, 271, pp. 1529–1535. doi: 10.1098/rspb.2004.2756.
Gardner, A., West, S. A. and Wild, G. (2011) 'The genetical theory of kin selection', *Journal of Evolutionary Biology*, 24, pp. 240–256. doi: 10.1111/j.1420-9101.2011.02236.x.
Gilbert, O. M. *et al.* (2007) 'High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants', *Proceedings of the National Academy of Sciences*, 104, pp. 8913–8917. doi: 10.1073/pnas.0702723104.
Giron, D. *et al.* (2004) 'Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition', *Nature*, 430, p. 676. doi: 10.1038/nature02721.
Giron, D., Pincebourde, S. and Casas, J. (2004) 'Lifetime gains of host-feeding in a synovigenic parasitic wasp', *Physiological Entomology*, 29, pp. 436–442. doi: 10.1111/j.0307-6962.2004.00414.x.
Grafen, A. (1984) 'Natural selection, kin selection and group selection', in *Behavioural Ecology: an Evolutionary Approach*. 2nd edn, pp. 62–84. doi: papers2://publication/uuid/3E0E8E8B-B12A-46D2-AEA0-2062D25A790C.
Grafen, A. (1985) 'A geometric view of relatedness', *Oxford Surveys in Evolutionary Biology*, 2, pp. 28–89.
Grafen, A. and Archetti, M. (2008) 'Natural selection of altruism in inelastic viscous homogeneous populations', *Journal of Theoretical Biology*, 252, pp. 694–710. doi: 10.1016/j.jtbi.2008.01.021.
Grbic, M., Ode, P. J. and Strand, M. R. (1992) 'Sibling Rivalry and Brood Sex-Ratios in Polyembryonic Wasps', *Nature*, 360, pp. 254–256. doi: Doi 10.1038/360254a0.
Griffin, A. S. and West, S. A. (2003) 'Kin Discrimination and the Benefit of Helping in Cooperatively Breeding Vertebrates', *Science*, 302, pp. 634–636. doi: 10.1126/science.1089402.
Griffin, A. S., West, S. A. and Buckling, A. (2004) 'Cooperation and competition in pathogenic bacteria', *Nature*, 430, pp. 1024–1027. doi: 10.1038/nature02744.
Hamilton, W. D. (1964) 'The genetical evolution of social behaviour I-II', *Journal of Theoretical Biology*, 7, pp. 1–52. doi: 10.1016/0022-5193(64)90038-4.
Hamilton, W. D. (1967) 'Extraordinary sex ratios', *Science*, 156, pp. 477–488. doi: 10.1126/science.156.3774.477.
Hamilton, W. D. (1970) 'Selfish and spiteful behaviour in an evolutionary model', *Nature*, 228, pp. 1218–1220. doi: 10.1038/2281218a0.
Hamilton, W. D. (1971) 'Geometry for the selfish herd', *Journal of Theoretical Biology*, 31, pp. 295–311. doi: 10.1016/0022-5193(71)90189-5.
Hamilton, W. D. (1975) 'Innate Social Aptitudes of Man: an Approach from Evolutionary Genetics', *Biosocial Anthropology*, 53, pp. 133–155. doi: no DOI, no URL.
Harvey, P. H. and Purvis, A. (1991) 'Comparative methods for explaining adaptations', *Nature*, 351, pp. 619–624. doi: 10.1038/351619a0.
Hatchwell, B. J. (2009) 'The evolution of cooperative breeding in birds: kinship, dispersal

and life history', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364, pp. 3217–3227. doi: 10.1098/rstb.2009.0109.

Hughes, W. O. H. *et al.* (2008) 'Ancestral monogamy shows kin selection is key to the evolution of eusociality', *Science*, 320, pp. 1213–1216. doi: 10.1126/science.1156108.

Inglis, R. F. *et al.* (2009) 'Spite and virulence in the bacterium Pseudomonas aeruginosa', *Proceedings of the National Academy of Sciences*, 106, pp. 5703–5707. doi: 10.1073/pnas.0810850106.

Innocent, T. M. *et al.* (2010) 'Competition between relatives and the evolution of dispersal in a parasitoid wasp', *Journal of Evolutionary Biology*, 23, pp. 1374–1385. doi: 10.1111/j.1420-9101.2010.02015.x.

Irwin, A. J. and Taylor, P. D. (2001) 'Evolution of altruism in stepping-stone populations with overlapping generations', *Theoretical Population Biology*, 60, pp. 315–325. doi: 10.1006/tpbi.2001.1533.

Johnstone, R. A. (2008) 'Kin selection, local competition, and reproductive skew', *Evolution*, 62, pp. 2592–2599. doi: 10.1111/j.1558-5646.2008.00480.x.

Johnstone, R. A. A. and Cant, M. A. A. (2008) 'Sex Differences in Dispersal and the Evolution of Helping and Harming', *The American Naturalist*, 172, pp. 318–330. doi: 10.1086/589899.

Johnstone, R. A. and Cant, M. A. (2010) 'The evolution of menopause in cetaceans and humans: The role of demography', *Proceedings of the Royal Society B: Biological Sciences*, 277, pp. 3765–3771. doi: 10.1098/rspb.2010.0988.

Kelly, J. K. (1992) 'Kin selection in density regulated populations', *Journal of Theoretical Biology*, 157, pp. 126–129. doi: 10.1016/S0022-5193(05)80663-0.

Komdeur, J. (1994) 'The effect of kinship on helping in the cooperative breeding Seychelles warbler (Acrocephalus sechellensis)', *Proceedings of the Royal Society B: Biological Sciences*, 256, pp. 47–52. doi: 10.1098/rspb.1994.0047.

Komdeur, J., Richardson, D. S. and Burke, T. (2004) 'Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness', *Proceedings of the Royal Society B: Biological Sciences*, 271, pp. 963–969. doi: 10.1098/rspb.2003.2665.

Kümmerli, R. *et al.* (2009) 'Limited dispersal, budding dispersal, and cooperation: An experimental study', *Evolution*, 63, pp. 939–949. doi: 10.1111/j.1558-5646.2008.00548.x.

Kuzdzal-Fick, J. J. *et al.* (2011) 'High relatedness is necessary and sufficient to maintain multicellularity in Dictyostelium', *Science*, 334, pp. 1548–1551. doi: 10.1126/science.1213272.

Lehmann, L. *et al.* (2007) 'Group selection and kin selection: Two concepts but one process', *Proceedings of the National Academy of Sciences*, 104, pp. 6736–6739. doi: 10.1073/pnas.0700662104.

Lehmann, L., Bargum, K. and Reuter, M. (2006) 'An evolutionary analysis of the relationship between spite and altruism', *Journal of Evolutionary Biology*, 19, pp. 1507–1516. doi: 10.1111/j.1420-9101.2006.01128.x.

Lehmann, L., Perrin, N. and Rousset, F. (2006) 'Population demography and the evolution of helping behaviors', *Evolution*, 60, pp. 1137–1151. doi: 10.1111/j.0014-3820.2006.tb01193.x.

Lehmann, L., Ravigné, V. and Keller, L. (2008) 'Population viscosity can promote the evolution of altruistic sterile helpers and eusociality', *Proceedings of the Royal Society B: Biological Sciences*, 275, pp. 1887–1895. doi: 10.1098/rspb.2008.0276.

Lehmann, L. and Rousset, F. (2010) 'How life history and demography promote or inhibit

the evolution of helping behaviours', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, pp. 2599–2617. doi: 10.1098/rstb.2010.0138.
Levin, S. R. and West, S. A. (2017) 'The evolution of cooperation in simple molecular replicators', *Proceedings of the Royal Society B: Biological Sciences*, 284, p. 20171967. doi: 10.1098/rspb.2017.1967.
Levin, S. and West, S. (2017) 'Kin Selection in the RNA World', *Life*, 7, p. 53. doi: 10.3390/life7040053.
Liao, X., Rong, S. and Queller, D. C. (2015) 'Relatedness, Conflict, and the Evolution of Eusociality', *PLoS Biology*, 13, p. e1002098. doi: 10.1371/journal.pbio.1002098.
Lion, S. (2013) 'Multiple infections, kin selection and the evolutionary epidemiology of parasite traits', *Journal of Evolutionary Biology*, 26, pp. 2107–2122. doi: 10.1111/jeb.12207.
Lion, S. and Gandon, S. (2009) 'Habitat saturation and the spatial evolutionary ecology of altruism', *Journal of Evolutionary Biology*, 22, pp. 1487–1502. doi: 10.1111/j.1420-9101.2009.01769.x.
Lion, S., Jansen, V. A. A. and Day, T. (2011) 'Evolution in structured populations: Beyond the kin versus group debate', *Trends in Ecology and Evolution*, 26, pp. 193–201. doi: 10.1016/j.tree.2011.01.006.
Lukas, D. and Clutton-Brock, T. (2012) 'Life histories and the evolution of cooperative breeding in mammals', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 2151–2156. doi: 10.1098/rspb.2012.1433.
Lukas, D. and Clutton-Brock, T. H. (2012) 'Cooperative breeding and monogamy in mammalian societies', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 2151–2156. doi: 10.1098/rspb.2011.2468.
May, R. M. (2004) 'Uses and Abuses of Mathematics in Biology', *Science*, 303, pp. 790–793. doi: 10.1126/science.1094442.
Mehdiabadi, N. J. *et al.* (2006) 'Kin preference in a social microbe', *Nature*, 442, pp. 881–882. doi: 10.1038/442881a.
Mitteldorf, J. and Wilson, D. S. (2000) 'Population viscosity and the evolution of altruism', *Journal of Theoretical Biology*, 204, pp. 481–496. doi: 10.1006/jtbi.2000.2007.
El Mouden, C. and Gardner, A. (2008) 'Nice natives and mean migrants: The evolution of dispersal-dependent social behaviour in viscous populations', *Journal of Evolutionary Biology*, 21, pp. 1480–1491. doi: 10.1111/j.1420-9101.2008.01614.x.
Nee, S., West, S. A. and Read, A. F. (2002) 'Inbreeding and parasite sex ratios', *Proceedings of the Royal Society B: Biological Sciences*, 269, pp. 755–760. doi: 10.1098/rspb.2001.1938.
Noh, S. *et al.* (2018) 'Genetic signatures of microbial altruism and cheating in social amoebas in the wild', *Proceedings of the National Academy of Sciences*, 115, pp. 3096–3101. doi: 10.1073/pnas.1720324115.
Ohtsuki, H. *et al.* (2006) 'A simple rule for the evolution of cooperation on graphs and social networks', *Nature*, 441, pp. 502–505. doi: 10.1038/nature04605.
Ostrowski, E. A. *et al.* (2015) 'Genomic Signatures of Cooperation and Conflict in the Social Amoeba', *Current Biology*, 25, pp. 1661–1665. doi: 10.1016/j.cub.2015.04.059.
Parker, G. A. and Maynard Smith, J. (1990) 'Optimality theory in evolutionary biology', *Nature*, 348, pp. 27–33. doi: 10.1038/348027a0.
Pen, I. and Weissing, F. J. (2000) 'Towards a unified theory of cooperative breeding: The role of ecology and life history re-examined', *Proceedings of the Royal Society B: Biological Sciences*, 267, pp. 2411–2418. doi: 10.1098/rspb.2000.1299.

Peña, J., Nöldeke, G. and Lehmann, L. (2015) 'Evolutionary dynamics of collective action in spatially structured populations', *Journal of Theoretical Biology*, 382, pp. 122–136. doi: 10.1016/j.jtbi.2015.06.039.

Perrin, N. and Lehmann, L. (2001) 'Is Sociality Driven by the Costs of Dispersal or the Benefits of Philopatry? A Role for Kin-Discrimination Mechanisms', *The American Naturalist*, 158, pp. 471–483. doi: 10.1086/323114.

Pfennig, D. W. and Collins, J. P. (1993) 'Kinship affects morphogenesis in cannibalistic salamanders', *Nature*, 362, pp. 836–838. doi: 10.1038/362836a0.

Pfennig, D. W., Collins, J. P. and Ziemba, R. E. (1999) 'A test of alternative hypotheses for kin recognition in cannibalistic tiger salamanders', *Behavioral Ecology*, 10, pp. 436–443. doi: 10.1093/beheco/10.4.436.

Pfennig, D. W., Sherman, P. W. and Collins, J. P. (1994) 'Kin recognition and cannibalism in polyphenic salamanders', *Behavioral Ecology*, pp. 225–232. doi: 10.1093/beheco/5.2.225.

Pollitt, E. J. G. *et al.* (2014) 'Cooperation, quorum sensing, and evolution of virulence in Staphylococcus aureus', *Infection and Immunity*, 82, pp. 1045–1051. doi: 10.1128/IAI.01216-13.

Popat, R. *et al.* (2015) 'Conflict of interest and signal interference lead to the breakdown of honest signaling', *Evolution*, 69, pp. 2371–2383. doi: 10.1111/evo.12751.

Queller, D. C. (1994) 'Genetic relatedness in viscous populations', *Evolutionary Ecology*, 8, pp. 70–73. doi: 10.1007/BF01237667.

Ratnieks, F. L. W. (1988) 'Reproductive Harmony via Mutual Policing by Workers in Eusocial Hymenoptera', *The American Naturalist*, 132, pp. 217–236. doi: 10.1086/284846.

Ratnieks, F. L. W., Foster, K. R. and Wenseleers, T. (2006) 'Conflict resolution in insect societies.', *Annual review of entomology*, 51, pp. 217–236. doi: 10.1146/annurev.ento.51.110104.151003.

Rautiala, P., Helanterä, H. and Puurtinen, M. (2014) 'Unmatedness Promotes the Evolution of Helping More in Diplodiploids than in Haplodiploids', *The American Naturalist*, 184, pp. 318–325. doi: 10.1086/677309.

Rodrigues, A. M. M. and Gardner, A. (2012) 'Evolution of helping and harming in heterogenous populations', *Evolution*, 66, pp. 2065–2079.

Rodrigues, A. M. M. and Gardner, A. (2013a) 'Evolution Of Helping And Harming In Heterogeneous Groups', *Evolution*, 67, pp. 2284–2298. doi: 10.1111/evo.12110.

Rodrigues, A. M. M. and Gardner, A. (2013b) 'Evolution of helping and harming in viscous populations when group size varies', *The American Naturalist*, 181, pp. 609–622. doi: 10.1086/670031.

Rogers, a R. (1990) 'Group Selection By Selective Emigration the Effects of Migration and Kin Structure.', *American Naturalist 1990 135 3 398413*, 135, pp. 398–413. doi: http://www.jstor.org/stable/2460163.

Rousset, F. (2004) *Genetic Structure and Selection in Subdivided Populations*. Princeton: Princeton University Press.

Rumbaugh, K. P. *et al.* (2012) 'Kin selection, quorum sensing and virulence in pathogenic bacteria', *Proceedings of the Royal Society B: Biological Sciences*, 279, pp. 3584–3588. doi: 10.1098/rspb.2012.0843.

Russell, A. F. and Hatchwell, B. J. (2001) 'Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate', *Proceedings of the Royal Society B: Biological Sciences*, 268, pp. 2169–2174. doi: 10.1098/rspb.2001.1790.

Sachs, J. L. *et al.* (2004) 'The Evolution of Cooperation', *The Quarterly Review of Biology*, 79, pp. 135–160. doi: 10.1086/383541.

Sharp, S. P. *et al.* (2005) 'Learned kin recognition cues in a social bird', *Nature*, 434, pp. 1127–1130. doi: 10.1038/nature03522.
Taylor, P. D. (1988) 'An inclusive fitness model for dispersal of offspring', *Journal of Theoretical Biology*, 13, pp. 363–378. doi: 10.1016/S0022-5193(88)80035-3.
Taylor, P. D. (1992a) 'Altruism in viscous populations - an inclusive fitness model', *Evolutionary Ecology*, 6, pp. 352–356. doi: 10.1007/BF02270971.
Taylor, P. D. (1992b) 'Inclusive fitness in a homogeneous environment', *Proceedings of the Royal Society B: Biological Sciences*, pp. 299–302. doi: 10.1098/rspb.1992.0118.
Taylor, P. D. and Frank, S. A. (1996) 'How to make a kin selection model', *Journal of Theoretical Biology*, 180, pp. 27–37. doi: 10.1006/jtbi.1996.0075.
Taylor, P. D. and Irwin, A. J. (2000) 'Overlapping generations can promote altruistic behavior', *Evolution*, 5, pp. 1135–1141. doi: 10.1111/j.0014-3820.2000.tb00549.x.
Traulsen, A. and Nowak, M. a (2006) 'Evolution of cooperation by multilevel selection.', *Proceedings of the National Academy of Sciences of the United States of America*, 103, pp. 10952–10955. doi: 10.1073/pnas.0602530103.
Trivers, R. L. and Hare, H. (1976) 'Haplodiploidy and the evolution of the social insects', *Science*, 191, pp. 249–263. doi: 10.1126/science.1108197.
Wenseleers, T., Hart, A. G. and Ratnieks, F. L. W. (2013) 'When Resistance Is Useless: Policing and the Evolution of Reproductive Acquiescence in Insect Societies', *The American Naturalist*, 164, pp. E154–E167. doi: 10.1086/425223.
Wenseleers, T. and Ratnieks, F. L. W. (2004) 'Tragedy of the commons in Melipona bees', *Proceedings of the Royal Society B: Biological Sciences*, 271, pp. S310–S312. doi: 10.1098/rsbl.2003.0159.
Wenseleers, T. and Ratnieks, F. L. W. (2006) 'Comparative Analysis of Worker Reproduction and Policing in Eusocial Hymenoptera Supports Relatedness Theory', *The American Naturalist*, 168, pp. E163–E179. doi: 10.1086/508619.
Wenseleers, T. and Ratnieks, F. L. W. (2006) 'Enforced altruism in insect societies', *Nature*, 444, p. 50. doi: 10.1038/444050a.
Wenseleers, T., Ratnieks, F. L. W. and Billen, J. (2003) 'Caste fate conflict in swarm-founding social Hymenoptera: An inclusive fitness analysis', *Journal of Evolutionary Biology*, 16, pp. 647–658. doi: 10.1046/j.1420-9101.2003.00574.x.
West, S. A. *et al.* (2001) 'Testing Hamilton's rule with competition between relatives', *Nature*, 409, pp. 510–513. doi: 10.1038/35054057.
West, S. A. *et al.* (2002) 'Sanctions and mutualism stability: Why do rhizobia fix nitrogen?', *Proceedings of the Royal Society B: Biological Sciences*, 269, pp. 685–694. doi: 10.1098/rspb.2001.1878.
West, S. A. (2009) *Sex Allocation*. Princeton: Princeton University Press.
West, S. A. and Buckling, A. (2003) 'Cooperation, virulence and siderophore production in bacterial parasites', *Proceedings of the Royal Society B: Biological Sciences*, 270, pp. 37–44. doi: 10.1098/rspb.2002.2209.
West, S. A., Griffin, A. S. and Gardner, A. (2007a) 'Evolutionary Explanations for Cooperation', *Current Biology*, pp. R661–R672. doi: 10.1016/j.cub.2007.06.004.
West, S. A., Griffin, A. S. and Gardner, A. (2007b) 'Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection', *Journal of Evolutionary Biology*, 20, pp. 415–432. doi: 10.1111/j.1420-9101.2006.01258.x.
Westneat, D. and Fox, C. W. (2010) *Evolutionary behavioral ecology*. Oxford: Oxford University Press.
Wild, G. (2011) 'Direct fitness for dynamic kin selection', *Journal of Evolutionary Biology*. doi: 10.1111/j.1420-9101.2011.02291.x.
Wild, G., Gardner, A. and West, S. A. (2009) 'Adaptation and the evolution of parasite

virulence in a connected world', *Nature*, 459, pp. 983–986. doi: 10.1038/nature08071.

Wild, G. and West, S. A. (2007) 'A Sex Allocation Theory for Vertebrates: Combining Local Resource Competition and Condition-Dependent Allocation', *The American Naturalist*, pp. E112–E128. doi: 10.1086/522057.

Yeh, A. Y. C. and Gardner, A. (2012) 'A general ploidy model for the evolution of helping in viscous populations', *Journal of Theoretical Biology*, pp. 297–303. doi: 10.1016/j.jtbi.2012.04.004.

Chapter 6: General discussion and conclusions

Each chapter of this thesis contains a discussion section relevant to each particular project. Here, I discuss only the broader themes relevant to the thesis as a whole and the research program upon which I have embarked. The immediate aim of this thesis was to begin building a theoretical body of work with which to understand why division of labour evolves in some cases and why it takes different forms in different species. As such, I first present how some of my results compare to the predictions from reproductive skew theory. The long-term aim is to link my research to the major evolutionary transitions framework. That is, I would like to understand how and why division of labour facilitates evolutionary transitions in individuality. To that end, I then outline here several key ways in which the work contained in this thesis may be extended toward its larger goals.

Comparison with predictions from reproductive skew theory

High reproductive skew occurs when only a few individuals in the social group monopolise reproduction (Vehrencamp, 1979; Johnstone, 2000). There are many reproductive skew models and their predictions are sometimes in opposing directions. For example, concession models tend to predict that higher relatedness leads to more reproductive skew, restraint models predict that higher relatedness leads to lower reproductive skew and compromise models predict that relatedness has little to no effect on reproductive skew (Johnstone, 2000; Reeve and Keller, 2001). Synthetic models predict each of these different outcomes in different areas of parameter space (Johnstone, 2000; Buston *et al.*, 2009).

Within the framework of my work, high reproductive skew occurs when: 1) the proportion of helpers to reproductives is higher; and 2) helpers are more specialised on cooperation and reproduce less. Across all of my work, I have found that more related social groups favour higher reproductive skew. This applies equally whether the helper is sterile or a generalist (chapter 2) and whether the benefits of cooperation are rivalrous, non-rivalrous, shared equally, preferentially directed, or synergistic (chapter 3). Consequently, my predictions are at odds with those of restraint and transaction models of reproductive skew. However, my work makes the same qualitative predictions as those from concession models.

The predictions from my models and from concession models are in broad agreement with empirical findings. For instance, empirical work has shown that relatedness correlates with reproductive skew in small colony social insects, that lower matedness favours cooperative breeding and the evolution of eusociality, and that subsocial group formation favours the evolution of multicellularity and potentially higher percentages of sterile cells (Reeve and Keller, 2001; Hughes *et al.*, 2008; Cornwallis *et al.*, 2010; Fisher, Cornwallis and West, 2013). However, an assessment of whether concession models or my work is the better explanatory framework for these patterns requires empirical work comparing which of their modelling assumptions are more reasonable. In particular, my work does not assume that reproductives control or enforce the fecundity of helpers.

Cheating and within-group competition

How does the presence of potential cheaters influence the evolution of division of labour? Division of labour is characterised by cooperative behaviours that provide benefits to social partners at a private cost (condition 2). Cheaters are individuals who enjoy the

benefits of this cooperation but do not contribute equitably to the pool of social benefits and thus pay a reduced private cost (Axelrod and Hamilton, 1981; Hauert and Doebeli, 2004; Doebeli and Hauert, 2005; Ross-Gillespie *et al.*, 2007; Gore, Youk and Van Oudenaarden, 2009; Ghoul, Griffin and West, 2014). For our purposes, a cheater may be an individual that is less likely to adopt a helping role, or whose helping phenotype contributes less than that of resident co-operators. Consequently, cheating lineages may sometimes outcompete labour dividers. If so, this can lead to several potential outcomes. Cheaters may displace labour dividers entirely and thus collapse cooperation, or an equilibrium frequency of cheaters to labour dividers may arise (Ross-Gillespie *et al.*, 2007; Gore, Youk and Van Oudenaarden, 2009). In either case, I would like to know how the presence of cheaters shapes the evolution of division of labour and its potential to drive transitions in individuality. If wholesale cheating is allowed, what is the effect on the patterns predicted by my division of labour model (Chapter 2)?

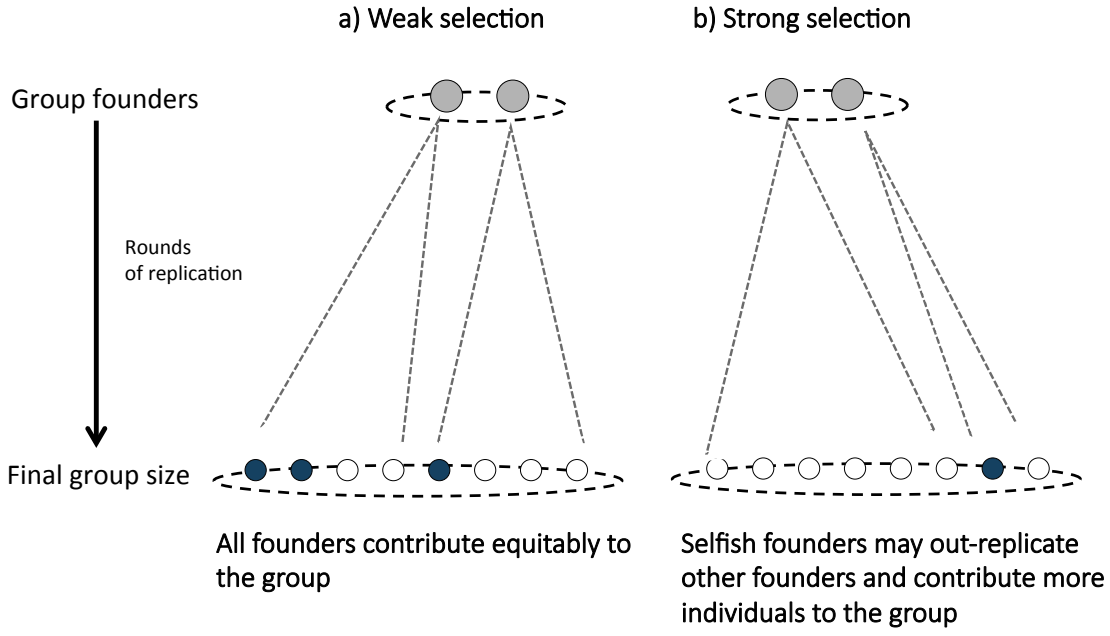


Figure 1: Cheating during the group growth phase. a) In my division of labour model (Chapter 2), I assumed weak selection. Therefore, any cheating founders will only contribute marginally less to cooperation compared to other founders and therefore there will be a negligible impact on the composition of groups by the end of the group growth phase. b) I would like to investigate the

consequence of allowing for strong selection. In this case, a cheating founder may not contribute to cooperation at all (uniform non-cooperation) and therefore may outcompete all other lineages during the group growth phase.

In particular, Chapter 2 only allowed a particular kind of cheating to influence the evolution of labour division. This is because the model assumed weak selection- that all mutations only lead to marginal differences in trait value. As such, division of labour equilibria were only ever shown to be stable to cheaters that were only slightly less likely to adopt a helping phenotype or whose helping phenotype invested only slightly less in cooperation. Critically, we did not consider stability to wholesale cheaters that never adopt a helping phenotype, ($(p = 1, q_1 = 0)$ or $(p = 0, q_2 = 0)$ or $(q_1 = q_2 = 0)$; uniform non-cooperation). Furthermore, we used the assumption of weak selection to make a key approximation about the composition of groups. Since any mutations only lead to small differences in fitness, we assumed that selection was negligible during the growth of groups and thus that there was an equal proportion of individuals in the group descended from each group founder (**Figure 1a**). However, if wholesale cheating is allowed, cheating lineages would be expected to have a large fitness advantage during the growth of groups and thus contribute disproportionately to the composition of groups by the time they reach their maximal size and before offspring dispersal (Axelrod and Hamilton, 1981; Hauert and Doebeli, 2004; Doebeli and Hauert, 2005; Ross-Gillespie *et al.*, 2007; Gore, Youk and Van Oudenaarden, 2009; Ghoul, Griffin and West, 2014) (**Figure 1b**).

Consequently, a future project could involve extending the division of labour model (Chapter 2) to allow for mutations of any size and allowing for selection during the growth of groups. Furthermore, we would need to track the possibility of evolutionary outcomes composed of an equilibrium frequency of both cooperating and cheating lineages (Ross-Gillespie *et al.*, 2007; Gore, Youk and Van Oudenaarden, 2009). All combined, this would

enable us to evaluate the influence of all forms of cheating on the potential evolution of division of labour. This would be directly compared to the predicted patterns in Chapter 2. I am particularly interested in the effect of relatedness in this case as maximal relatedness (clonal in asexual systems; singly-mated in sexual systems) may be the only scenario in which wholesale cheaters cannot outcompete co-operators at all during the group growth phase. It is possible then that this effect may help to explain evolutionary patterns that suggest that maximal relatedness is a necessary condition for evolutionary transitions to multicellularity and eusociality (Hughes *et al.*, 2008; Boomsma, 2009; Fisher, Cornwallis and West, 2013).

Mutual dependence and the evolution of obligate groups.

How does division of labour facilitate fraternal evolutionary transitions? Some groups may be facultative (**Figure 2b** or **2d**) such that they only form in response to certain environmental and ecological conditions (Bourke, 2011; Fisher, Cornwallis and West, 2013). Consequently, these groups are a transient demographic structures, existing only for part of the life-time of its constituent individuals or for only a few generations. When the conditions that favoured the formation of groups cease to exist, the constituent individuals return to their free-living state (**Figure 2a**). Examples, include the formation of clumps by unicellular algae in response to predation or the formation of *Dycostelium discoideum* slime moulds in scarce resource conditions (Strassmann, Zhu and Queller, 2000; Kapsetaki, Fisher and West, 2016; Kapsetaki, Tep and West, 2017).

An evolutionary transition in individuality occurs when otherwise free-living individuals can no longer survive in isolation and so evolve to form obligate groups (**Figure 2c** or **2e**) (Bourke, 2011; West *et al.*, 2015). Because this is an irreversible process, it means that the

interests of the constituent individuals are then aligned in such a way that survival and proliferation of the group is indistinguishable from individual level evolutionary success (Gardner and Grafen, 2009). We would like to know the role that division of labour plays in the formation of obligate groups. Namely, is the evolutionary transition from state **Figure 2d** to **Figure 2e** more likely than that from **Figure 2b** to **Figure 2c**?

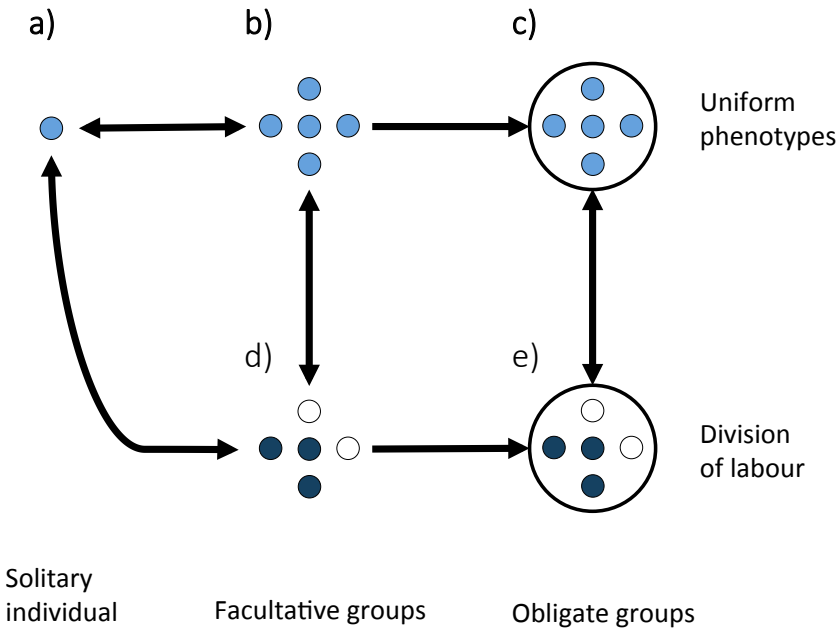


Figure 2: Division of labour and evolutionary transitions in individuality. In certain environmental conditions, a solitary individual (a) may facultatively form a group with other individuals (b). In some cases, individuals in this transient structure may engage in uniform cooperative behaviours (b) or they may engage in a facultative division of labour (d). An evolutionary transition in individuality (c) and (e) occurs when individuals in the group become so dependent on within-group cooperation that they can no longer survive as solitary individuals (a) and thus adopt an obligate group life cycle (c) and (e). We hypothesise that division of labour facilitates the transition from facultative groups (d) to obligate groups (e).

Our theoretical understanding is that this is the case. When individuals specialise and divide labour, they become more dependent upon each other such that they survive less well when on their own (Bourke, 2011; West *et al.*, 2015; Cooper and West, 2018). A tipping point may arise when they become so mutually dependent that individuals are

favoured to remain in a group configuration regardless of whether the initial conditions that favoured group formation persist. However, this theory is conceptual and verbal in nature. Consequently, I would like to explore whether the argument is corroborated by mathematical work grounded in evolutionary theory.

In Chapter 2, the model was agnostic as to whether the groups form through a facultative or obligate process. Instead, I solved for the environmental and demographic conditions that favour division of labour (**Figure 2d** or **2e**) over uniform behaviours (**Figure 2b** or **2c**). Whether these external conditions are faced every generation for all individuals (obligate) or only every few generations (facultative) is not considered.

I propose here a model that tracks all of these possibilities. I would develop a model that: 1) allows for two different environmental conditions, one favouring groups, the other favouring independent living; 2) tracks the evolution of a tendency to form groups in either environment; 3) allows to evolve the ability to divide labour, conditional on being in a group; and 4) includes a metabolic cost to plasticity. It is likely that this model will not be analytically solvable. As such, I will employ dynamic, individual based simulation whenever necessary.

In this way, I will be able to investigate whether obligate group formation is more likely to evolve when individuals divide labour. Once again, it will be interesting to examine the effect on relatedness to see whether obligate groups only every form when relatedness is maximal as suggested by empirical results (Hughes *et al.*, 2008; Boomsma, 2009; Fisher, Cornwallis and West, 2013).

Division of labour and group fitness

What is the relationship between individual-level fitness and group fitness when helpers and reproductives specialise into their respective roles? Michod (2006) compares each form of fitness and finds that reproductive division of labour leads to a 'transfer of fitness' whereby individual-level fitness is decreased in order to increase group fitness. However, this analysis does not directly compare individual and group fitness in the same demographic conditions. Instead the fitness of the labour divided group is compared with the individual-level fitness of specialised individuals outside of the group structure (Michod, 2006). Needless to say, there are no benefits to reproductive specialisation outside of the group and it is unsurprising that individual level fitness decreases in this artificial case. If the appropriate comparison is made where individuals are grouped in both cases, then it can easily be shown that individual-level fitness and group fitness are the same (in Michod's model).

However, this does raise the broader question: when is division of labour favoured by both individual-level selection and group-level selection? In the theoretical literature, most works consider division of labour as a strategy that may optimise group fitness (Michod, 2006; Tannenbaum, 2007; Willensdorfer, 2009; Rueffler, Hermisson and Wagner, 2012; Solari, Kessler and Goldstein, 2013). In fact, developing a contrasting individual-level (inclusive fitness) approach was a motivation for my work in Chapter 2. As such, determining in what cases the predictions of both approaches should align would be of conceptual interest. Previous, more general, theory suggests that inclusive fitness and group fitness are both maximised when there is no conflict within groups (maximal relatedness) or when all conflict is supressed (Gardner and Grafen, 2009). We anticipate

that these predictions would be corroborated if a specific division of labour comparison were made.

Genetic division of labour

In Chapters 2, 3 and 4, I have considered reproductive division of labour between individuals who share the same genotype but who have adopted different phenotypic roles (**Figure 3a**; helper and reproductive). In Chapter 3, I evaluated when this kind of division of labour (phenotypic) should evolve over uniform behaviours and, in Chapter 4, I considered the favourability of two different ways in which individuals of the same genotype might adopt their respective roles (environmental signalling vs. random specialisation).

Another form of division of labour that I have not considered is the division of labour between individuals with different genotypes (**Figure 3b**) (Wahl, 2002). In this case, each phenotype is hard wired by different genotypes (**Figure 3b**) rather than being plastically expressed by a common genotype (**Figure 3a**). For example, some empirical work has found that there can be a genetic component to worker-queen specialisation in eusocial insect societies (Schwander *et al.*, 2010).

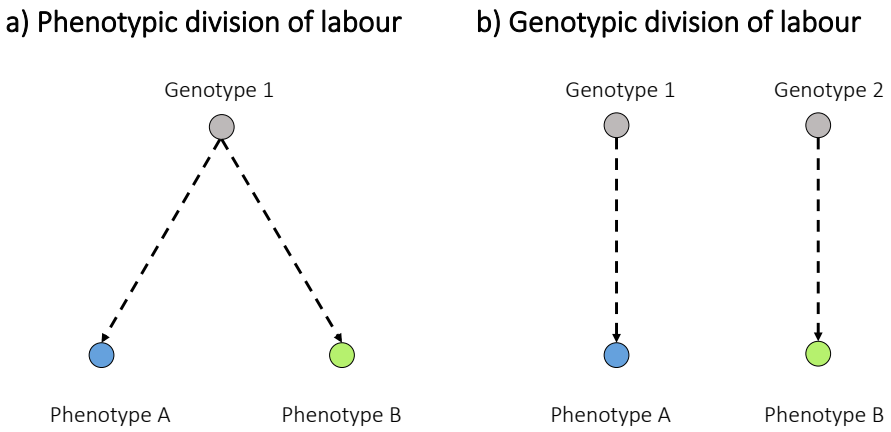


Figure 3: Phenotypic and genetic division of labour. a) Phenotypic division of labour occurs when the different individuals in the division have the same genotype but adopt distinct roles by the expression of different genetic pathways. b) genetic division of labour occurs when the different individuals have distinct genotypes and thus each have homogenous expression of genetic pathways.

This suggests that theory could be developed to make general predictions about the conditions that favour each of the three mechanisms for dividing labour. All theory up to this point has only considered a certain form of division of labour and has only considered pairwise comparisons between mechanisms. For instance, my work in Chapter 4 showed when coordination by environmental information should be favoured over random specialisation only for reproductive division of labour. Wahl (2002) has shown when random specialisation should be favoured over genetic division of labour but only for non-reproductive division of labour. I propose here a more general framework for both reproductive and non-reproductive division of labour that compares all three mechanisms concurrently. This would involve analytical, pairwise comparisons combined with a broader simulation that competes all mechanisms. In particular, I would consider the effect of different number of founding lineages as this will impact the relatedness of groups, which will be an important factor when considering the favourability of genetic division of labour.

Critically, division of labour between different genetic lineages of the same species (one performing a task A and the other performing a task B) may be a good first approximation of division of labour between individuals of different species, particularly if asexual reproduction is assumed. Consequently, the non-reproductive division results of this particular project may be relevant to understanding the role that division of labour plays in egalitarian transitions in individuality (Queller, 1997; Bourke, 2011).

Conclusion

Division of labour takes many forms and is found across the tree of life, at all levels of biological organisation (Bourke, 2011). Furthermore, when individuals divide labour, they become dependent upon one another and this can drive evolutionary transitions to higher levels of individuality (Bourke, 2011; West *et al.*, 2015). As such, a deeper understanding of the forces that favour and shape division of labour will not only help us to explain a diverse social behaviour but will also help us to understand why some forms of life have become so complex. The theoretical work of this thesis is but a humble foray into a wide field where much progress has already been made but where there nevertheless remain many questions with which to grapple.

References

Axelrod, R. and Hamilton, W. D. (1981) 'The Evolution of Cooperation', *Evolution*, 211, pp. 1390–1396. doi: 10.1086/383541.

Boomsma, J. J. (2009) 'Lifetime monogamy and the evolution of eusociality', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, pp. 3191–3207. doi: 10.1098/rstb.2009.0101.

Bourke, A. F. G. G. (2011) *Principles of Social Evolution*, *Principles of Social Evolution*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780199231157.001.0001.

Buston, P. M. *et al.* (2009) 'Reproductive skew and the evolution of conflict resolution : a synthesis of transactional and tug-of-war models', *Behavioral Ecology*, 20, pp. 672–684. doi: 10.1093/beheco/arp050.

Cooper, G. A. and West, S. A. (2018) 'Division of labour and the evolution of extreme specialization', *Nature Ecology and Evolution*. Springer US, 2, pp. 1161–1167. doi: 10.1038/s41559-018-0564-9.

Cornwallis, C. K. *et al.* (2010) 'Promiscuity and the evolutionary transition to complex societies', *Nature*, 466, pp. 969–972. doi: 10.1038/nature09335.

Doebeli, M. and Hauert, C. (2005) 'Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game', *Ecology Letters*, 8, pp. 748–766. doi: 10.1111/j.1461-0248.2005.00773.x.

Fisher, R. M., Cornwallis, C. K. and West, S. A. (2013) 'Group formation, relatedness, and the evolution of multicellularity', *Current Biology*, 23, pp. 1120–1125. doi: 10.1016/j.cub.2013.05.004.

Gardner, A. and Grafen, A. (2009) 'Capturing the superorganism: A formal theory of group adaptation', *Journal of Evolutionary Biology*, 22, pp. 659–671. doi: 10.1111/j.1420-9101.2008.01681.x.

Ghoul, M., Griffin, A. S. and West, S. A. (2014) 'Toward an evolutionary definition of cheating', *Evolution*, 68, pp. 318–331. doi: 10.1111/evo.12266.

Gore, J., Youk, H. and Van Oudenaarden, A. (2009) 'Snowdrift game dynamics and facultative cheating in yeast', *Nature*, 459, pp. 253–256. doi: 10.1038/nature07921.

Hauert, C. and Doebeli, M. (2004) 'Spatial structure often inhibits the evolution of cooperation in the snowdrif ...', pp. 2–5. doi: 10.1038/nature02422.1.

Hughes, W. O. H. *et al.* (2008) 'Ancestral monogamy shows kin selection is key to the evolution of eusociality', *Science*, 320, pp. 1213–1216. doi: 10.1126/science.1156108.

Johnstone, R. A. (2000) 'Models of reproductive skew: A review and synthesis', *Ethology*, 106, pp. 5–26. doi: 10.1046/j.1439-0310.2000.00529.x.

Kapsetaki, S. E., Fisher, R. M. and West, S. A. (2016) 'Predation and the formation of multicellular groups in algae', *Evolutionary Ecology Research*, 17, pp. 651–669.

Kapsetaki, S. E., Tep, A. and West, S. A. (2017) 'How do algae form multicellular groups?', *Evolutionary Ecology Research*, 18, pp. 663–675.

Michod, R. E. (2006) 'The group covariance effect and fitness trade-offs during evolutionary transitions in individuality', *Proceedings of the National Academy of Sciences*, 103, pp. 9113–9117. doi: 10.1073/pnas.0601080103.

Queller, D. C. (1997) 'Cooperators since life began', *Q Rev Bio*, 72, pp. 184–188.

Reeve, H. R. and Keller, L. (2001) 'Tests of reproductive skew models', *Annual review of entomology*, 46, pp. 347–385.

Ross-Gillespie, A. *et al.* (2007) 'Frequency dependence and cooperation: Theory and a test with bacteria', *Evolution*, 170, pp. 2315–2325. doi: 10.1111/j.1558-5646.2009.00723.x.

Supplementary materials 1. Defensive group formation and the evolution of multicellularity

Defensive Group Formation and the Evolution of Multicellularity

Guy Alexander Cooper and Stuart Andrew West

Abstract

The evolution of multicellularity represents one of the major transitions for life on Earth, where groups of independent individuals joined together to form a new higher level organism. The first step in the evolution of multicellularity is the formation of a cooperative group of cells. Given that clumping can lead to increased competition for resources, why would natural selection favour cells that group together? We show, theoretically, that the threat of predators can favour clumping, even when this leads to increased competition for resources within groups. We find that clumping is more likely to be favoured when: (i) the strength of predation is high; (ii) the cost of producing substances to stick cells together is low; (iii) the effectiveness of clumping as an evasion tactic is intermediate; (iv) competition costs are low and either increase dramatically before plateauing or are linear with respect to clump size. We suggest that variation in how facultatively multicellular species form clumps, such as lumps versus filaments, may reflect variation in the factors favouring clump formation.

Keywords: major evolutionary transitions, clumping, predation, facultatively multicellular species

Introduction

The evolution of life on Earth has involved approximately eight major evolutionary transitions in individuality, where individuals that could previously replicate independently join to form a more complex life form (Leigh, 1991; Maynard Smith and Szathmáry, 1995; Queller, 2000; Bourke, 2011). For example, genes formed genomes, cells formed multicellular organisms, and animals

formed eusocial societies. The first step in all of these transitions is individuals joining together to form a cooperative group (Bourke, 2011; West *et al.*, 2015). Here we are concerned with why individual cells joined together to form cooperative cell-groups. The transition to multicellularity has occurred over twenty-five times across the breadth of the tree of life, leading to organisms that range in complexity from clumps of algae to the gamut of differentiated splendour in the animal kingdom (Maynard Smith and Szathmáry, 1995; Grosberg and Strathmann, 2007).

A number of hypotheses have been suggested for why cells should clump together, each of which could be important in different taxa. These hypotheses include that clumps are better at dispersing, cooperating, foraging for and storing resources, or avoiding predators (Stanley, 1973; Boraas *et al.*, 1998; Kerszberg and Wolpert, 1998; Kirk, 1998; Szathmary and Wolpert, 2003; Velicer and Yuen-tsu, 2003; Grosberg and Strathmann, 2007; Koschwanez *et al.*, 2011, 2013; Santorelli *et al.*, 2013; Claessen *et al.*, 2014; Biernaskie and West, 2015). Here we address the hypothesis that clumping provides a defence against predators, because clumps of cells may be too big for predators to consume. This mechanism is thought to be important in several bacteria and algae species, including some where individuals form multicellular clumps facultatively in response to the presence of predators (reviewed by Fisher *et al*). These species include, among many others, the green algae, *Chlorella vulgaris*, the cyanobacterium, *Microcystis aeruginosa*, and the non-photosynthetic bacterium, *Comamonas acidovorans* (Boraas *et al.*, 1998; Hahn *et al.*, 1999; Ha *et al.*, 2004; Yang *et al.*, 2009).

Whilst the idea that predation favours multicellularity is intuitively appealing, there are a number of potential problems. How will any benefits of avoiding predation be balanced against the increased resource competition that results from clumping together? If clumping occurs by cells sticking together, then why are clumps not invaded by cheats that avoid the cost of producing sticky substances but can still be stuck to? What kinds of benefit and cost function shapes are required for this hypothesis to work? Given that unrelated cells could potentially stick together in clumps, how is selection for clumping influenced by the relatedness between interacting cells?

Our aim is to address these questions through a theoretical examination of how predation influences the evolution of cooperative groups. We consider the evolution of a trait, the production of a sticky substance, which leads to cells becoming stuck together in clumps through an aggregation process. This trait carries a metabolic cost to the individual producing it, a cost of increased

resource competition to all cells in the clump, and the potential benefit of predator avoidance to all cells in the clump. We examine a relatively simple scenario, to elucidate the key underlying selective forces, that could apply to algae and bacteria, or more widely.

Methods

Population Structure and Life History

We presume a haploid, asexual population of very large size that reproduces with non-overlapping generations. To facilitate analytical tractability, we take the common step of assuming an island model in which cells are divided into neighbourhoods that each contain equal numbers of cells. Each neighbourhood is comprised of the individuals that will interact with each other socially, such that each individual in a neighbourhood may clump with all the other individuals in its neighbourhood but not with individuals from different neighbourhoods.

We assume that the proclivity of the cells to clump is determined by the amount of a sticky substance the cells produce. For a focal cell of an arbitrary neighbourhood, we define the variable y as the amount of resources that the cell invests in the production of the substance. This phenotypic trait value is defined on the interval $[0, 1]$, where $y = 0$ corresponds to no production of the sticky substance and $y = 1$ corresponds to the production of the maximum amount of the substance of which the cell is capable. We, in turn, define z as the average investment in the substance across all cells in the focal cell's neighbourhood and $\hat{z}$ as the average investment across the entire population.

We assume that cells that produce more sticky substance (with higher y values) are not only more likely to stick to other cells but are also more likely to end up in bigger clumps of cells. We denote the predator-evasion or survival benefit provided to a focal cell as $E(y, z) \in [0, 1]$, where for generality at the moment we presume that E depends both on the focal cell's investment in the production of the sticky substance as well as the average investment of cells in its neighbourhood. Whether a cell survives predation and is able to reproduce will not only depend how well clumped it is but also on how this clumping compares with the degree of cell clumping throughout the population. Consequently, we assume that the relative survivability of the focal cell is $E(y, z)/E(\hat{z}, \hat{z})$. We assume that the threat of predation is constant over time and space.

The surviving population at the end of the life-cycle is replaced in its entirety by its spawn, where

the number of offspring will be equal to the parental population size before the predation effects. We model the specific fecundity of a cell that survived predation as $1 - F(y, z)$, where $F \in [0, 1]$ quantifies the expected resource costs to the cell throughout its life cycle. These resource costs come from two sources. Firstly, the production of the sticky substance results in a baseline resource cost to the cell. Secondly, costs are engendered as a result of being in a clump due to competition for local nutrients and light uptake (if the cells are photosynthetic). Once again, fecundity is actually determined relative to the rest of the population and so we model the relative fecundity of the focal cell as $(1 - F(y, z))/(1 - F(\hat{z}, \hat{z}))$ to reflect this. Offspring inherit the phenotypic trait values of their parent and disperse such that individuals are sorted randomly into neighbourhoods at the end of the life-cycle, independently of the parent generation population structure.

Kin Selection Analysis

We follow the standard kin selection methodology of Taylor and Frank (Taylor and Frank, 1996, 2010; Frank, 1998; Taylor *et al.*, 2007; Rousset, 2013). The fitness, W, of a focal cell in an arbitrary neighbourhood is the product of its relative fecundity and relative survivability, giving:

$$W = \frac{1 - F(y, z)}{1 - F(\hat{z}, \hat{z})} \times \frac{E(y, z)}{E(\hat{z}, \hat{z})}, \tag{1}$$

where E and F are monotonically increasing functions of their variables. Equation 1 makes clear that there will be a trade-off between resources invested into survival versus resources invested into reproduction. An increased investment in the sticky substance will lead to a higher survivability, but a reduced fecundity.

The survival benefit of being in a group will depend upon the size of the group. In order to increase the tractability of our model, we make the simplifying assumption that the group size in which our focal cell finds itself depends only upon the average production of the sticky substance in the neighbourhood, z, and not upon the individual production of the substance by the cell, y. This assumption will be especially valid at larger group sizes, where the focal cell makes up a small fraction of the group. We assume thus that $E(y, z) = b(z)$, where b is the survival benefit of being in a group where individuals in the neighbourhood are producing an average amount z of the sticky

substance. We require that $0 \leq b(z) \leq 1$ for all values of z.

We model the reproduction cost of producing the sticky substance as an additive function of the cost the cell experiences from sticky substance production and the cost experienced by the cell due to increased resource competition from being in a group. Specifically, that $F(y, z) = c_0(y) + c_1(z)$, where c_0 quantifies the resources lost to the cell by production of the sticky substance and c_1 quantifies the increased resource competition due to clumping. We have the restriction that $0 \leq c_0(y) + c_1(z) \leq 1$ for all permissible values of y and z. This gives the individual fitness expression as

$$W = \frac{1 - (c_0(y) + c_1(z))}{1 - (c_0(\hat{z}) + c_1(\hat{z}))} \times \frac{b(z)}{b(\hat{z})}. \tag{2}$$

We use the fitness of the focal cell defined in Equation 2 to solve for the Evolutionary Stable Strategy (ESS) value of y, which we denote as y* (Maynard Smith and Price, 1973). The ESS in our model will be the value of the production of the sticky substance such that, if all individuals in the population shared this value as their phenotypic trait, no mutant allele with a small perturbation in that value will be able to invade the population. We calculate the ESS by solving for the equilibrium value of the selection gradient,

$$\frac{\partial W}{\partial y} + \frac{\partial W}{\partial z} R = 0, \tag{3}$$

where R is the average relatedness of the individual to its neighbourhood and the partial derivatives are evaluated at $y = z = \hat{z} = y^*$ (Taylor and Frank, 1996; Frank, 1998, 2010). Using Equations 2 and 3, we find that the equilibrium values of the considered trait satisfy the condition:

$$\frac{c_0'}{1 - (c_0 + c_1)} = R\Big(\frac{b'}{b} - \frac{c_1'}{1 - (c_0 + c_1)}\Big). \tag{4}$$

Presuming the equilibrium value is indeed a fitness-maxima, this shows that the value of the trait will be an ESS if the marginal cost to the individual, $\mathrm{C} = c_0'/(1 - (c_0 + c_1))$, equals the

marginal benefit to the group, $B = (b'/b) - c_1'/(1 - (c_0 + c_1))$, weighted by the average relatedness of the focal individual to the group, R. This gives us a form of Hamilton's rule (Hamilton, 1964), $RB - C = 0$, with R measuring relatedness to the whole group, including the actor (Pepper, 2000). If we set c_1 to be identically zero, we reduce the model to a general public goods model where there are no public costs and where public benefits are balanced against only private costs, in which case our Equation 4 simplifies to the special case of equation 3 in Frank (Frank, 2010).

Fitness Functions

In order to proceed, we need to assume specific forms for c_0, c_1 and b. To maintain flexibility, we assume a family of functions that can model both accelerating and diminishing group costs and benefits to clumping. The elements of this family are characterized by the parameter vector, $(\alpha, \beta, \gamma, \theta, \lambda, \mu)$, and by the functional forms,

$$\begin{aligned} c_0(y) &= \alpha y \\ c_1(z) &= \beta z^{\theta\lambda} \\ b(z) &= \gamma + (1-\gamma) z^{\theta\mu}. \end{aligned}$$

All parameters are given in in Table 1 along with their constraints and physical interpretations.

We assume that the individual resource cost of sticky substance production, c_0, is a linear function of how much substance is produced. The parameter α controls the slope of this response, with the cost of sticky substance production increasing as α increases. In addition, we have the parameter constraint that $\alpha \in [0, 1]$ so that c_0 is also constrained to the unit interval for all values of y.

The group cost and benefit functions, c_1 and b, depend on the size of the focal cell's clump, which we have assumed depends upon the group average production of the sticky substance, z. We assume that the size of the focal cells group is z^θ, where $\theta > 0$, and we have rescaled such that when $z^\theta = 0$ the focal cell is not in a clump at all and when z^θ equals its maximum permissible value of 1 we consider the cell to be in a clump of the maximum possible size (the patch is fully clumped). The relationship between the average production of the sticky substance and group size

can therefore be linear ($\theta = 1$), show diminishing returns ($\theta < 1$) or accelerating returns ($\theta > 1$; Fig. 1 a).

The λ and β parameters determine how group size influences resource competition, where $\lambda \geq 0$ and $\beta \in [0, 1]$. The relationship between group size and the resource competition cost of being in a group will be linear when $\lambda = 1$, diminishing when $\lambda < 1$, and accelerating when $\lambda > 1$. The relationship between group size and resource cost is scaled by β, which represents the cost when group size is at its maximum value ($z = 1$; Fig. 1 b). The sum of α and β controls the maximum amount that the focal cell's resources can be depleted by and this gives the additional constraint that $\alpha + \beta \leq 1$, ensuring that the general cost function, F, is appropriately defined for all values of y and z.

The γ and μ terms determine how group size influences the likelihood of predation. The parameter γ, where $\gamma \in [0, 1]$, equals the likelihood of survival when group size is zero ($z = 0$; Fig. 1 c), and thus $1 - \gamma$ can be considered a measure of the strength of predation in the system. The relationship between group size and likelihood of survival can be linear ($\mu = 1$), diminishing ($\mu < 1$) or accelerating ($\mu > 1$; Fig 1. d). The parameter μ can be interpreted as the effectiveness of clumping as a predator evasion tactic, where low μ signifies that clumping is comparatively effective.

By substituting these functional forms and their derivatives in Equation 4 we find that the equilibrium production of sticky substance y^* is the solution of the following equation for the variable $\hat{z}$:

$$\begin{aligned} \hat{z}^{\theta(\lambda+\mu)-1}[R\beta\theta(1-\gamma)(\mu+\lambda)] + \hat{z}^{\theta\mu}[\alpha(1-\gamma)(1+R\theta\mu)] \\ +\hat{z}^{\theta\mu-1}[-R(1-\gamma)\theta\mu] + \hat{z}^{\theta\lambda-1}[R\beta\theta\lambda\gamma] + \alpha\gamma = 0. \end{aligned} \tag{5}$$

Parameter Assumptions

We present results for parameter assumptions based upon the biology of algal-predator and bacteria-predator systems. We presume that α equals β and thus that the amount by which the cell's resources are depleted is at maximum due equally to its own production of the sticky substance as

well as to the limitation of resource uptake caused by clumping. We assume that this maximum can only deplete half of the cell's specific resources and thus we set $\alpha = \beta = 0.25$. We presume, for the moment, that there is a moderate degree of predation in the system and so set $\gamma = 0.5$. In turn, we assume that the transformation of the production of stickiness into the clump size is linear and thus that $\theta = 1$.

We also presume that the response curves for the group cost and benefit due to the clump size are both diminishing. For costs, there is likely to be an initially steep cost to clumping as the number of cells in the clump increases, but this increasing cost will then diminish with group size as the focal cell will already be shaded from resources. Considering benefits, the additional benefit against predators from increased group size will diminish when the group size increases beyond the number that the predator can successfully predate. We set $\lambda = \mu = 0.5$ to represent these diminishing qualities. Unless otherwise stated, we also presume a moderate degree of relatedness between the focal cell and its neighbourhood, with $R = 0.5$.

Results

We solved for the ESS numerically, with the parameter values give previously, by solving for the root, $\hat{z}$, in Equation 5 using the bisection method. We also checked that the ESS values determined are indeed maxima in the fitness landscape by considering the second derivative of fitness using the Taylor-Frank approach (Taylor and Frank, 1996; Frank, 1998, 2010). In addition to the results that we present here, we undertook a local exploration of parameter space as a sensitivity analysis, and found qualitatively similar patterns when each parameter, in turn, was perturbed from the above assumptions. The parameters α and β were considered over the range $0.15 - 0.35$, the parameters γ, λ, and μ were considered over the range $0.4 - 0.6$, the parameter θ from $0.9 - 1.1$, and the relatedness, R, was considered from $0.2 - 0.8$.

The Benefits of Clumping

We found that the ESS investment in sticky substance (y^*) increased with both: (a) an increasing relatedness between the members of the social group (R), and (b) an increasing strength of predation ($1-\gamma$) (Fig. 2 a). Thus we find broadly that defensive group formation is favoured at high predation

levels and high local relatedness. These predictions arise because they increase the survival benefit of increased clumping ($b(z)$), and because that benefit will be shared with closer relatives. Higher relatedness also means that the increased resource competition from clumping will be shared with closer relatives ($c_1(z)$), but our analyses show how this can be outweighed by the increased benefit to relatives.

We found that the parameter μ, which determined the shape of the relationship between group size and survival benefit, had a domed relationship with the ESS production of sticky substance (Fig. 2 b). The greatest production of sticky substance was at intermediate values of μ. At very small values of μ, the marginal benefit of increased clumping (b'/b) is initially large but then rapidly diminishes (Fig. 1 d), such that only a small amount of clumping is favoured. At very large values of μ, the marginal benefit of increased clumping (b'/b) is very small until there is an appreciable amount of clumping (Fig. 1 d), such that the benefits are outweighed by the costs. Consequently, the marginal benefits of increased clumping are greatest at intermediate values of μ where an increased clumping leads to a sufficiently increased benefit of clumping.

The Costs of Clumping

We found that the ESS production of sticky substance (y*) decreases as both the magnitude of the cost of producing the sticky substance (α) and the magnitude of competition for resources that results from clumping (β) increase (Fig. 3 a). These predictions arise because they increase the private ($c_o(y)$) and the group ($c_1(z)$) costs of clumping.

We found that the curvature of the group cost, λ, can have an inverse domed relationship with the ESS production of sticky substance (Fig. 3 b). The lowest ESS production of sticky substance was at intermediate values of λ, with y* increasing at higher or lower values of λ. When λ is small, any marginal group costs (c_1'/c_1) of increasing group size quickly level off (Fig. 1 b), allowing larger group sizes to be favoured. When λ is large, any marginal group costs (c_1'/c_1) of increasing group size are very small until very large group sizes are obtained (Fig. 1 b), which also allows large group sizes to be favoured. Consequently, the marginal costs of increased clumping are greatest at intermediate values of λ, where an increased clumping leads to a consistently increasing group cost of clumping ($c_1(z)$; Fig. 1 b).

Discussion

We found that multicellular group formation can be favoured as a defense against predation. Group formation is more likely to be favoured when: (1) there is a higher relatedness among interacting cells (higher R); (2) the threat of predation is high (higher $1-\gamma$); (3) the effectiveness of clumping as a predator avoidance tactic is intermediate (intermediate μ); (4) the costs of both producing a sticky substance to form groups, and the cost of increased resource competition are lower (lower α and β respectively); (5) the cost of increased competition for resources either increases quickly to a plateau or linearly with respect to groups size ($\lambda \approx 0$ or 1).

Why is multicellular group formation stable in response to the potential pressure from cheats, who avoid the cost of producing sticky substance, but can still get stuck in a group? We found the production of sticky substance required a sufficiently high relatedness within the group. Consequently, cheats are not favoured, because their production of less sticky substance would lead to both themselves and their relatives being in smaller groups, where predation is higher. However, the relatedness term in our model is the relatedness of the focal individual to the whole group, and so also includes relatedness to self (Pepper, 2000). Specifically, if an individual is in a group with $n-1$ individuals to which they are related by r, then the whole group relatedness is given by $R = [(n-1)r+1]/n$. This suggests that clumping can even be favoured with non-relatives, if the predation pressure is sufficiently strong.

What predictions does our model make that could be applied to or tested in the real world? First, we predict that clumping will be more likely when predators are more common (Fig. 2 a; higher $1-\gamma$), and when an increase in group size can have a substantial influence on the rate of predation (Fig. 1 b; intermediate μ). Second, we predict that, if the predation pressure varies over time, such that the benefits of group formation only outweigh the costs when predators are especially common, then individuals will be selected to form colonies facultatively, in response to the presence of predators. Such facultative group formation appears to occur in a number of algal and bacteria species (reviewed by Claessen *et al.*, 2014; reviewed by Fisher *et al*).

Third, individuals can be favoured to form groups in ways that keep relatives together, to increase the kin selected benefits of helping relatives avoid predators (Fig. 2 a). One way to do this is via sticking to parental cells, and forming clonal groups (Fisher *et al*, 2013). Another way is via

kin discrimination (Strassmann *et al*, 2011). Kin discrimination has not yet been observed when forming groups to better avoid predators. Nonetheless, even if kin discrimination is not possible, and interactions are only with non-relatives, clump formation may still be favoured, if the direct benefits of predator avoidance are enough to outweigh the costs.

Fourth, methods of group formation will be favoured that miminise competition for resources ($c_1(z)$). This could explain the filamentous clumping geometries that have been observed in both algal and bacterial species. This prediction of clumping to miminise direct interaction is in the exact opposite direction from another hypothesis for multicellular group formation. Specifically, if clumps are favoured to better share the benefits of excreted factors, then we would expect direct interactions to be maximised, and hence more ball shaped lumps, as occurs in yeast (Koschwanez *et al*, 2011, 2013; Biernaskie and West, 2015). This suggests that the different types of group formation, which can be found in different facultatively multicellular species, may represent different factors driving clump formation. A comparative approach could be used to test this prediction, comparing across groups where the benefit of group formation varies. Once multicellular groups have formed, the next step is to understand why cells differentiate to different types, producing a division of labour and more complex organisms (Jeanson *et al.*, 2007; Michod, 2007; Gavrilets, 2010; Rossetti *et al.*, 2010).

References

Biernaskie, J.M. and West, S.A. 2015. Cooperation, clumping and the evolution of multicellularity. *Proc. Roy. Soc. B: Biol. Sci.* **282**: 20151075.

Boraas, M.E., Seale, D.B., and Boxhorn, J.E. 1998. Phagotrophy by a flagellate selects for colonial prey: a possible origin of multicellularity. *Evol. Ecol.* **12**: 153-164.

Bourke, A.F.G. 2011. *Principles of Social Evolution.* Oxford: Oxford University Press.

Claessen, D., Rozen, D.E., Kuipers, O.P., Sogaard-Andersen, L., and van Wezel, G.P. 2014. Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. *Nature Rev.*

Microbiol. **12**: 115-124.

Fisher, R.M., Cornwallis, C.K., and West, S.A. 2013. Group formation, relatedness, and the evolution of multicellularity. *Curr. Biol.* **23**: 1120-1125.

Fisher, R.M., Bell, T. and West, S.A. 2015. Multicellular group formation in response to predators in the alga Chlorella vulgaris. *J. Evol. Biol.* doi: 10.1111/jeb.12804

Foster, W.A. 1990. Experimental evidence for the effective altruistic colony defense against natural predators by soldiers of the gall-formin aphid Pemphigus spyrothecae (Hemiptera: Pemphigidae). *Behav. Ecol. Sociobio.* **27**: 421-430.

Frank, S.A. 1998. *Foundations of Social Evolution.* Princeton, NJ: Princeton University Press.

Frank, S.A. 2010. A general model of the public goods dilemma. *J. Evol. Biol.* **23**: 1245-1250.

Gavrilets, S. 2010. Rapid transition towards the division of labor via evolution of developmental plasticity. *PLoS Comput Biol.* **6**: e1000805-e1000805.

Grosberg, R.K. and Strathmann, R.R. 2007. The evolution of multicellularity: A minor major transition? *Ann. Rev. Ecol. Evol. Syst.* **38**: 621-654.

Ha, K., Jang, M.H., and Takamura, N. 2004. Colony formation in planktonic algae induced by zooplankton culture media filtrate. *J. Freshwater Ecol.* **19**: 9-16.

Hahn, M.W., Moore, E.R., and Hfle, M.G. 1999. Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. *Appl. Environ. Microb.* **65**: 25-35.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. *J. Theor. Biol.* **7**: 17-52.

Jeanson, R., Fewell, J. H., Gorelick, R., and Bertram, S. M. 2007. Emergence of increased division of labor as a function of group size. *Behav. Ecol. Sociobiol.* **62**: 289-298.

Kerszberg, M., Wolpert, L. 1998. The origin of metazoa and the egg: a role for cell death. *J. Theor. Biol.* **193**: 535-537.

Kirk, D.L. 1998. *Volvox: Molecular-Genetic Origins of Multicellularity and Cellular Differentiation.* Cambridge: Cambridge University Press.

Korb, J., Buschmann, M., Schafberg S., Liebig, J., Bagneres, A.G. 2012. Brood care and social evolution in termites. *Proc. Roy. Soc. B* **279**: 2662-2671.

Koschwanez, J.H., Foster, K.R., Murray, A.W. 2011. Sucrose utilization in budding yeast as a model for the origin of undifferentiated multicellularity. *PLoS Biol.* **9**: 1703.

Koschwanez, J.H., Foster, K.R., Murray, A.W. 2013. Improved use of a public good selects for the evolution of undifferentiated multicellularity. *Elife* **2**: e00367.

Leigh, E.G., Jr. 1991. Genes, bees and ecosystems: The evolution of a common interest among individuals. *Trends Ecol. Evol.* **6**: 257-262.

Lurling, M. and Van Donk, E. 2000. Grazer-Induced Colony Formation in Scenedesmus: Are There Costs to Being Colonial? *Oikos* **88**: 111-118.

Maynard Smith, J., and Price, G.R. 1973. The Logic of Animal Conflict. *Nature* **246**: 15.

Maynard Smith, J., and Szathmáry, E. 1995. *The Major Transitions in Evolution.* New York: Oxford University Press.

Michod, R.E. 2007. Evolution of individuality during the transition from unicellular to multicellular life. *Proc. Nat. Acad. Sci. USA* **104**: 8613-8618.

Pepper, J. W. 2000. Relatedness in trait group models of social evolution. *J. Theor. Biol.* **206**: 355-368.

Pike, N., Whitfield, J.A., Foster, W.A. 2007. Ecological correlates of sociality in Pemphigus aphids, with partical phylogeny of genus. *BMC Evol. Biol.* **7**: 185.

Queller, D.C. 2000. Relatedness and the fraternal major transitions. Philos. Trans. R. Soc. London B Biol. Sci. **355**: 1647-1655.

Rousset, F. 2013. *Genetic Structure and Selection in Subdivided Populations (MPB-40).* Princeton, NJ: Princeton University Press.

Rossetti, V., Schirrmeister, B.E., Bernasconi, M.V., and Bagheri, H.C. 2010. The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J. Theor. Biol.* **262**: 23-34.

Santorelli, L.A., Kuspa, A., Shaulsky, G., Queller D.C., Strassmann, J.E. 2013. A new social gene in Dictyostelium discoideum, chtB. *BMC Evol. Biol.* **13**: 4.

Shultz, S., Opie, C., Atkinson, Q.D. 2011. Stepwise evolution of stable sociality in primates. *Nature* **479**: 219-222.

Stanley, S.M. 1973. An ecological theory for the sudden origin of multicellular life in the late Precambrian. *Proc. Nat. Acad. Sci. USA* **70**: 1486-1489.

Strassmann, J.E., Gilbert, O.M., and Queller, D.C. 2011. Kin discrimination and cooperation in microbes. *Annu. Rev. Microbio.* **65**: 349-367.

Szathmary, E., Wolpert L. 2003. The transition from single cells to multicellularity. In: *Genetic and Cultural Evolution of Cooperation* (Hammerstein, P. ed.), pp. 271-290. MIT Press.

Taylor, P.D., and Frank, S.A. 1996. How to make a kin selection model. *J. Theor. Biol.* **180**: 27-37.

Taylor, P.D., Day, T., and Wild, G. 2007. Evolution of cooperation in a finite homogeneous graph. *Nature*, **447**: 469-472.

Velicer, G.J., and Yuen-tsu, N.Y. 2003. Evolution of novel cooperative swarming in the bacterium Myxococcus xanthus. *Nature* **425**: 75-78.

West, S.A., Fisher, R. M., Gardner A, Kiers E.T. 2015. Major evolutionary transitions in individuality. *Proc. Natl. Acad. Sci.*: 201412402.

Yang, Z., Kong, F., Yang, Z., Zhang, M., Yu, Y., and Qian, S. 2009. Benefits and costs of the grazer-induced colony formation in Microcystis aeruginosa. *Ann. Limnol.-Int. J. Lim.* **45**: 203-208.

Ackowledgments: We thank Jay Biernaskie, Roberta M. Fisher, and Stephanie Kapsetaki for their comments and discussion. G. A. C. is supported by the Engineering and Physical Sciences Research Council. S. A W. is supported by the European Research Council.

Figure Legends

Figure 1: Cost and benefit functions. (a) The relationship between the group average production of the sticky substance, z, and group size, z^θ, for different values of the shape parameter, θ. (b) The relationship between group size, z^θ, and the group cost of increased resource competition, c_1, for different costs of clumping, β. Here λ is fixed at 0.5 and so the response curves are diminishing. (c) The relationship between group size, z^θ, and group benefit due to clumping, b, for varying strengths of predation, $1-\gamma$. Here μ is fixed at 0.5 and so the response curves are diminishing. (d) The relationship between the group size, z^θ, and group benefit due to clumping, b, with a varying effectiveness of clumping as a predator evasion tactic, μ. γ is fixed at 0.5 and thus the strength of predation is moderate.

Figure 2: The benefits of group formation. The influence on the ESS production of the sticky substance y^* of (a) relatedness, R, and (b) the effectiveness of clumping as a predator evasion tactic, μ. Results are plotted for varying strengths of predation, $1-\gamma$. A higher production of sticky substance is favoured when interacting cells are more highly related and when predation is important (a), and when the effectiveness of clumping as predator evasion tactic is intermediate (b). The plots in (b) are truncated at higher μ values because there is no real-valued root to the equilibrium equation in this regime.

Figure 3: The costs of group formation. The influence on the ESS production of the sticky substance y^* of (a) the magnitude of private costs, α, and (b) the curvature of the group cost, λ. Results are plotted for varying magnitudes of group cost to competition, β. A higher production of sticky substance is favoured when the resource costs due to production of the substance and increased competition are small (a), and when group cost increases either linearly with respect to group size or dramatically at first before plateauing (b).

Table 1: Parameters of theoretical model with their constraints and physical interpretations.

Parameters	Constraints	Interpretation
α	$\alpha \in [0, 1]$	Scale of cost of individual production of stickiness
β	$\beta \in [0, 1]$ and $\alpha + \beta \leq 1$	Scale of group cost to clumping
γ	$\gamma \in [0, 1]$	$1 - \gamma$ quantifies strength of predation.
θ	$\theta > 0$	Effectiveness of production of sticky substance as a clumping mechanism
λ	$\lambda > 0$	Curvature of group cost to clumping
μ	$\mu > 0$	Effectiveness of clumping as a predator evasion tactic

Figure 1:

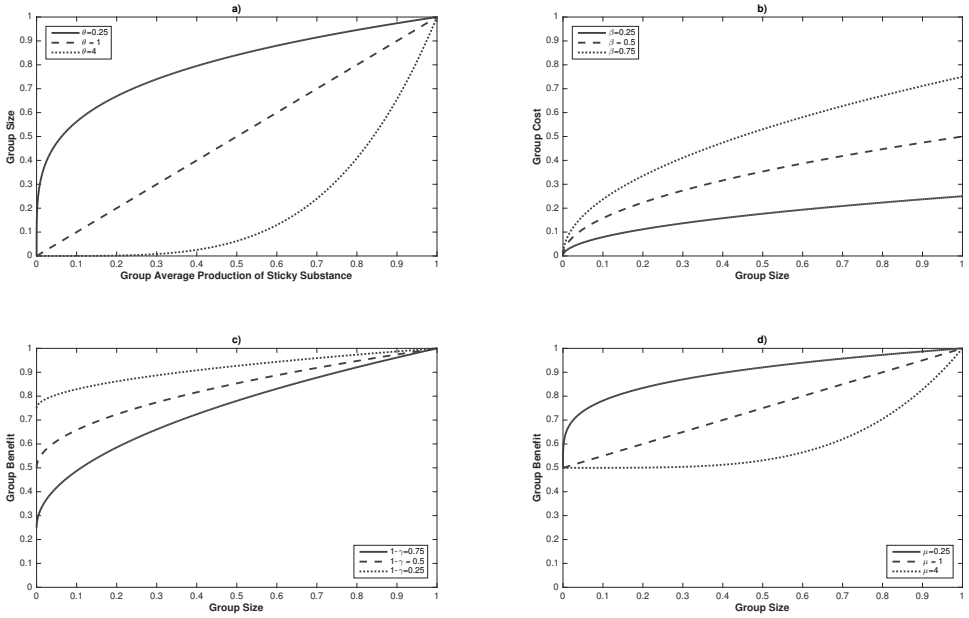


Figure 2:

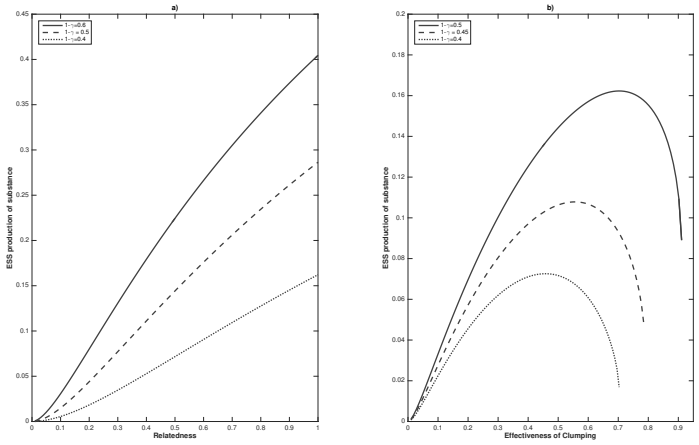
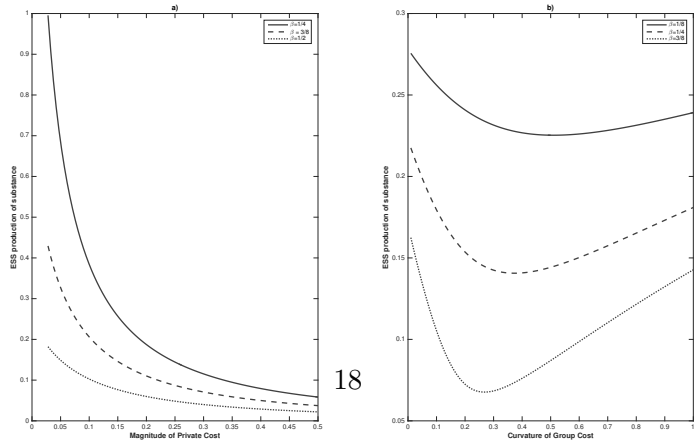


Figure 3:



Supplementary materials 2. Division of labour in microorganisms: an evolutionary perspective (published paper)

OPINION

Division of labour in microorganisms: an evolutionary perspective

Stuart A. West and Guy A. Cooper

Abstract | The division of labour, whereby individuals within a group specialize in certain tasks, has long been appreciated as central to the evolution of complex biological societies. In recent years, several examples of division of labour in microorganisms have arisen, which suggests that this strategy may also be important in microbial species. In this Opinion article, we explore the set of conditions that define division of labour and propose that cooperation between different phenotypes is a defining feature of division of labour. Furthermore, we discuss how clarifying what constitutes division of labour highlights key evolutionary questions, including what form division of labour takes and why it is favoured by natural selection.

Microbial cells in a population often show extreme phenotypic variation, which can arise through several mechanisms (BOX 1). For example, when *Escherichia coli* cells are growing in a batch culture, a proportion of the cells are in a transient non-growing state, termed persister cells, whereas the rest of the cells are growing normally[1]. A standard explanation for this phenotypic variation is that it represents a bet-hedging strategy, whereby different phenotypes are more successful in different environments, and therefore the fitness of cells is increased by varying the phenotype[2,3]. In the case of *E. coli*, the persister phenotype promotes survival under conditions of environmental stress, such as the presence of antibiotics[4].

However, phenotypic variation often cannot be explained as bet-hedging. These other examples seem to involve the specialization of some cells in a population to carry out cooperative tasks that benefit other cells that do not carry out the cooperative task (FIG. 1A). For example, in populations of *Bacillus subtilis* at stationary phase grown in liquid medium, a proportion of the cells produce and excrete proteases that degrade proteins in the environment into smaller peptides, which can be used as nutrient sources[5]. As the proteases and their degradation products freely diffuse, the production of proteases is beneficial for the local cell population and not just for the protease-secreting cells. In this Opinion article, we propose that cooperation between different phenotypes is a defining feature of division of labour[3,6,7].

Determining whether examples of phenotypic variation represent division of labour raises new questions. Why would natural selection favour division of labour? Why would it be beneficial for just a proportion of cells in a given population to carry out a trait, such as protease production, rather than all cells carrying out that trait at a lower rate? If the division of labour is cooperative, why can lineages that produce a lower proportion of the cooperative phenotype (that is, cheats)[8] not invade the population, which would eventually lead to the loss of division of labour? Why is division of labour favoured by natural selection in certain environments, for certain tasks, but not others? Or why are different mechanisms, such as phenotypic noise or environmental cues[2] (BOX 1), used to promote phenotypic variation in different situations?

A better understanding of division of labour may also provide insights into the evolution of other biological processes, including virulence and multicellularity. The division of labour seems to be central to the success and virulence of pathogenic species, such as *Cryptococcus gattii*, which is the causative agent of fungal meningitis. During infection, host reactive oxygen species, an essential part of the host immune response, induce a tubular mitochondrial phenotype in a proportion of *C. gattii* cells[9]. When phagocytes engulf cells of both types, the cells with tubular mitochondria are able to protect the normal cells and increase their intracellular proliferation[9]. Finally, the division of labour between cells has had a crucial role in the evolution of multicellularity, and therefore to elucidate how complex life on earth has evolved, we need to understand why division of labour is favoured in microorganisms[10–12].

In this Opinion article, we explore what constitutes division of labour within a microbial species. We propose a set of conditions that define division of labour and discuss whether several previously described examples of phenotypic plasticity represent adaptive division of labour. A precise definition is crucial, because imprecise definitions and ambiguity can obscure the fundamental problems and impede conceptual unification[13,14]. We outline key questions in the study of division of labour in microorganisms, focusing on what division of labour is, why it is favoured by natural selection and what forms it can take.

Complementary approaches

Before defining division of labour, it is useful to distinguish between mechanistic and evolutionary approaches to studying traits or behaviours[15]. The mechanistic (proximate) approach is to ask questions about how traits are controlled, such as what are the molecular or genetic mechanisms that control a particular trait (how questions; BOX 1). The evolutionary (ultimate) approach is to ask questions about the fitness consequences of that trait, and why it has been favoured by natural selection (why questions).

The majority of previous work on phenotypic heterogeneity has been mechanistic — our aim in this Opinion article is to ask evolutionary questions[2,3,6,7,16,17]. The crucial point

Box 1 | **Mechanisms of phenotypic variation**

There are at least six possible mechanisms for generating phenotypic variation within a species[2,17]. There can be overlap between these mechanisms.

Genetic differences. Standing genetic variation or mutations can lead to different phenotypes[27].

Epigenetics. Different phenotypes could be maintained by epigenetic inheritance, such as DNA methylation, which leads to a correlation in phenotype across generations[67].

Noise. If random fluctuations in the biochemical reactions of the cell (noise) are coupled with a gene network that amplifies small differences in reaction levels, this can lead to phenotypic variation[2,17]. For example, the phenotypic variation between *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells that do and do not express type III secretion system 1 (*ttss-1*), or exoprotease secretion in *Bacillus subtilis*, both arise from noise[5,22].

Signalling. In cyanobacteria, such as *Anabaena* spp., signalling peptides that are exported from one cell induce the development of a neighbouring cell into a nitrogen-fixing heterocyst[16]. This produces a regular pattern of heterocysts every relatively fixed number of vegetative cells among the filamentous colony. This number can vary from approximately 4–15 cells, depending on the species.

Environment. Variation can be generated by environmental cues. For example, in cyanobacteria, in addition to the role of signalling, nitrogen stress can lead to a higher proportion of cells that develop into heterocysts[16].

Condition dependence. Variation can be generated by differences in cell condition. In *Volvox carteri*, a series of asymmetric cell divisions, during early embryonic development, lead to the generation of small and large cells, which develop into soma and germ, respectively[68,69]. This process involves a gene that ancestors used to reduce reproduction during stressful conditions, and is being co-opted to produce a non-reproductive phenotype[68,70,71]. Variation in condition can also be dependent on the environment; for example, during nutrient-depleted conditions, cells in poorer condition might be more likely to become a non-reproductive altruist.

here is that these two approaches are complementary and not competing. Mechanistic answers cannot be given for evolutionary questions and vice versa, but an understanding from one perspective can aid the other perspective[18]. For example, an evolutionary approach can suggest when we might find different mechanisms in different species, whereas a mechanistic understating of what factors stimulate phenotypic heterogeneity can help us understand why that heterogeneity is favoured.

What is division of labour?

We define the division of labour as when cooperating individuals specialize to carry out specific tasks. This requires three conditions: individuals carry out different tasks (phenotypic variation); some individuals carry out cooperative tasks that benefit other individuals (cooperation); the division of tasks provides an inclusive fitness benefit to all of the individuals involved (adaptation).

We and others[7] emphasize the importance of division of labour as being cooperative. A behaviour or trait is cooperative when it benefits another individual and has been selected for, at least partially, because of this benefit[14].We emphasize cooperation because we are interested in cases in which individuals are working together, and have been selected to divide tasks, to the benefit of all individuals (see Supplementary information S1,S2 (boxes)). Therefore, division of labour represents a social adaptation across several individuals.

The second and third conditions (cooperation and adaptation) distinguish division of labour from cases in which phenotypic variation has arisen from self-interested traits, such as diversification to exploit different niches, or when one phenotype evolves to exploit another. We focus on inclusive fitness because it is our most general description of Darwinian fitness — natural selection favours traits that lead to an increase in inclusive fitness[19–21] (BOX 2). Consequently, if we are interested in whether a social trait, such as division of labour, can be favoured, we examine the inclusive fitness consequences. Our definition makes no claim as to whether a population must be clonal, although we discuss how clonality can influence whether and what form of division is favoured.

Examples of division of labour. During infection of a vertebrate host, a subpopulation of *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells remains in the gut lumen to reproduce, whereas other cells invade the gut tissue and express the type III secretion system 1 (*ttss-1*), which triggers an inflammatory response that eliminates competing bacteria from different species[22,23] (FIG. 1A). As the cells that enter the gut tissue are killed by the host immune system, this represents an altruistic cooperative behaviour, which is costly to the invading cells but benefits the cells that remained in the gut lumen (FIG. 1B; see Supplementary information S3 (box)). Another example of altruistic division of labour is provided by the fruiting bodies of slime moulds, such as *Dictyostelium discoideum*[24], in which non-viable stalk cells hold up and help disperse the viable spore cells. Similarly, spore cells also exist in the fruiting bodies of *Myxococcus xanthus*[25]. These examples are all analogous to the division of labour between germ cells and soma cells in multicellular species, in that some cells give up any opportunity to reproduce, to help reproductive cells[10].

Not division of labour. Our definition excludes several examples of phenotypic variation that are not cooperative and hence not division of labour. Under liquid culture conditions, *Pseudomonas fluorescens* exhibits different phenotypes, including 'smooth', 'wrinkly spreader' and 'fuzzy spreader' (REF. 26). This diversification represents the specialization of different lineages to exploit different niches: smooth phenotypes inhabit the liquid phase; wrinkly spreader phenotypes form a mat at the air–broth interface; and fuzzy spreader phenotypes inhabit the less aerobic environment at the bottom of the broth. These different phenotypes do not benefit each other cooperatively, and so this diversification does not represent division of labour. Similarly, various phenotypes are also observed in *P. fluorescens* colonies growing on agar, which can be explained by the self-interest of the different phenotypes[27].

Our definition also excludes cases in which cells can be divided between different lineages, whereby some lineages exhibit cooperative behaviour and others do not carry out, or carry out less of, the cooperative tasks. For example, *Pseudomonas aeruginosa* cells produce and secrete siderophores that scavenge iron from the environment. The benefits of iron scavenging are shared between the local cells, and so this is a cooperative behaviour[28]. However, lineages evolve, both in laboratory broth cultures and in the lungs of humans with cystic fibrosis, that produce less or no siderophores[29,30]. These lineages seem to act as cheats, which exploit the siderophores that are produced by other cells[8]. In this example, the cheats gain a selfish fitness benefit, but decrease the fitness of the lineages that produce

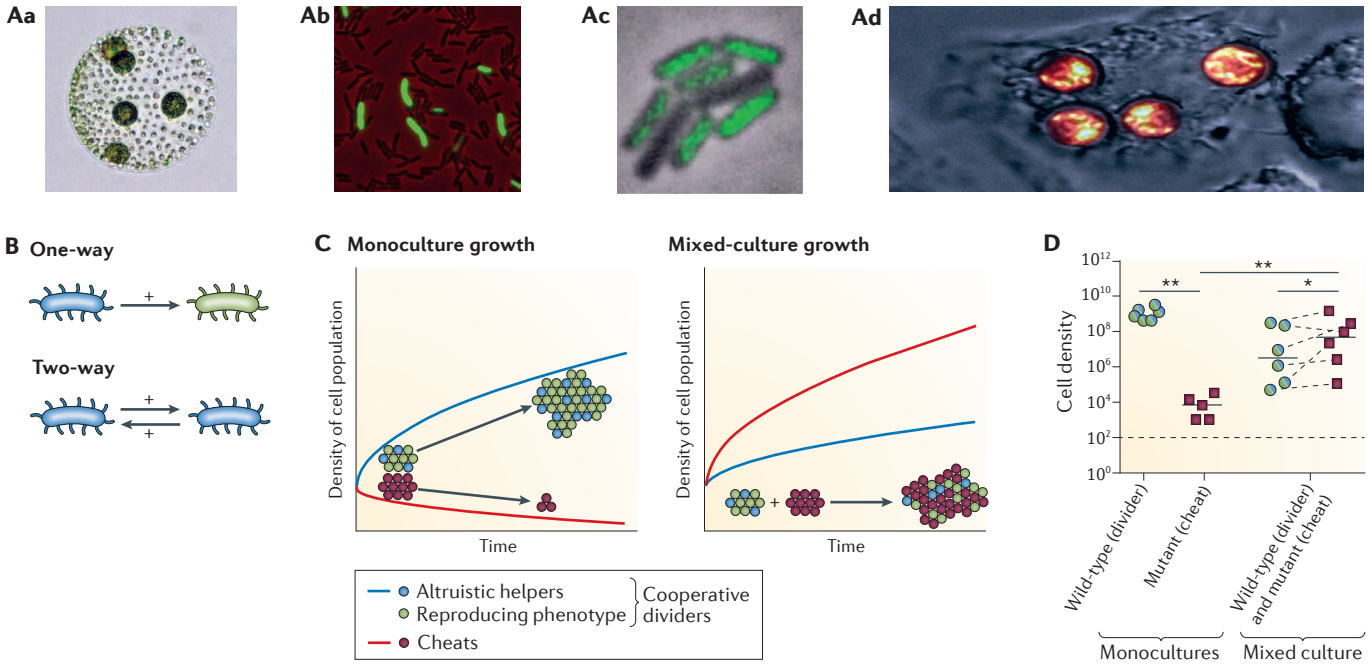


Figure 1 | **Division of labour. A** | Potential examples of division of labour. **Aa** | Phenotypic variation in *Volvox carteri*, which forms large germ cells and small soma cells. **Ab** | Some *Bacillus subtilis* cells (green) produce and excrete proteases that degrade proteins in the environment into smaller peptides. These peptides can be used as nutrient sources by *B. subtilis* cells and cells that do not produce and secrete the protease (grey). **Ac** | During infection, a subpopulation of *Salmonella enterica* subsp. *enterica* serovar Typhimurium cells (green) remains in the gut lumen to reproduce, whereas other cells (grey) invade the gut tissue and express the type III secretion system 1 (*ttss-1*), which triggers an inflammatory response that eliminates competing bacteria from different species. **Ad** | During infection, host reactive oxygen species induce a tubular mitochondrial phenotype in a proportion of *Cryptococcus gattii* cells (mitochondria are shown in yellow). **B** | Division of labour can involve either cooperation in one direction, whereby individuals of one phenotype help another phenotype (top panel; usually altruistically), or cooperation in both directions, whereby individuals of each phenotype cooperate and help the other (bottom panel; possibly to their mutual benefit; see Supplementary information S3, S5 (boxes)). **C** | Division of labour can be demonstrated by growing strains that do and do not carry out the putatively cooperative trait in both monocultures and mixed cultures. We consider: a cooperative strain, with division of labour, in which cells develop into both the altruistic helping phenotype (blue cells) and the reproducing phenotype (green cells); and a cheat strain, in which all cells develop into the reproducing phenotype (red cells). In monocultures (left panel), the cooperative strain, with division of labour, grows at a faster rate than a cheat strain. By contrast, in mixed cultures, the cheats are predicted to grow at a faster rate, because non-cooperative cheats can exploit the benefits that are provided by the altruistic helping phenotype. **D** | An example of this predicted pattern is provided by data from two strains of *S.* Typhimurium — the wild-type with division of labour (that is, cells that do and do not express *ttss-1*, which triggers an inflammatory response), and an *ahilD* mutant, which does not trigger inflammation. The wild-type exhibits an increased fitness when grown in monoculture, as measured by cells per gram of faeces, but decreased fitness when grown in a mixed culture, as the mutant benefits from the wild-type. Part **Aa** is adapted with permission from REF. 76, Springer. Part **Ab** is adapted from REF. 5. Part **Ac** is adapted from REF. 77. Image in part **Ad** courtesy of R. May, University of Birmingham, UK. Part **D** is adapted from REF. 23, Nature Publishing Group.

more siderophores. This contrasts with the examples of division of labour that are discussed above, in which all individuals benefit. In particular, in those examples, the helping and reproductive phenotypes are expressed conditionally, and therefore the helping phenotype is able to gain an inclusive fitness benefit from aiding its reproducing relatives. The contrast here is between an adaptation to exploit others (cheating), rather than an adaptation to help others (cooperative division of labour). An analogous example of cheating is lineages that do not produce or respond to quorum sensing molecules[31,32].

These examples show that division of labour requires more than the production of a novel 'joint' phenotype or the ability to carry out a task, which would not be possible without phenotypic diversity. For example, the different morphological variants of *P. fluorescens* can thrive in the liquid media of a beaker because of the production of various phenotypes[26]. These other types of phenotypic diversity are very interesting and result from different selection pressures. For example, cheats arise from conflict and not cooperation, and they do not require the efficiency benefits that are discussed below.

How to demonstrate division of labour?

First, it must be shown that there is phenotypic variation, with different individuals specializing to carry out different tasks. Second, it must be established that this division is cooperative and provides a fitness benefit to all of the cells that are involved.

Considering the example of *S.* Typhimurium discussed above, the differential expression of *ttss-1* in invading and non-invading cells represents phenotypic variation[22], but is this variation cooperative? To test this, strains that carry out the trait can be grown in both monocultures and mixed cultures with genetically manipulated strains that carry out less of, or do not carry out, the trait[18]. This design makes use of the fact that strains with less division of labour, and which carry out less of the potentially cooperative trait, would act as cheats[8]. If the trait is cooperative and provides a benefit to others, then cells that carry out the trait grow best as monocultures (cooperators outperform cheats), whereas cells that do not carry out the trait would grow best in mixed cultures[7,18] (cheats can exploit cooperators; FIG. 1C).

Box 2 | **Natural selection and adaptation**

Natural selection favours genes that are better at being transferred to the next generation[72]. However, researchers often talk about natural selection in the context of individual behaviour, leading to individuals that maximize their fitness. The formal justification for considering natural selection at the individual level is that genes that increase fitness will accumulate, and hence natural selection, through gene dynamics, will generate organisms that behave as if they are trying to maximize their fitness[72,73]. Thus, the gene and individual approaches are not competing, they are flip sides of the same coin — gene dynamics lead to the maximization of individual fitness.

Genes can influence their transmission to the next generation, not only by influencing the reproductive success of the individual they are in, but also by influencing the reproductive success of other individuals that carry the same gene. Hamilton[19] showed that natural selection will lead to individuals that behave as if they are maximizing not their personal reproductive success, but what he called 'inclusive fitness'. Inclusive fitness is the sum of fitness that is obtained directly, through reproduction, and indirectly through influencing the reproduction of relatives. Indirect fitness must be weighted according to relatedness, which is a statistical measure of the genetic similarity between individuals.

Hamilton's theory is often discussed in terms of kin selection and Hamilton's rule[19]. Hamilton's rule shows that an altruistic trait, such as becoming a sterile stalk cell in a fruiting body, will be favoured when $rB–C>0$ (where C is the fitness cost of carrying out the trait, B is the fitness benefit to other individuals, and r is the genetic relatedness to the individuals that receive the benefit). The most common ways for interacting cells to be related are by either limited dispersal keeping relatives together, or mechanisms of kin discrimination, which allow individuals to preferentially interact with relatives[19]. This approach emphasizes how we can explain division of labour, and especially altruistic division of labour, by examining the inclusive fitness, or kin selected, consequences.

Division of labour is often discussed as benefiting the population or community, with cells behaving analogous to a multicellular organism. It is useful to ask whether this is justified. More formally, we can ask when would gene dynamics lead to individuals that are trying to maximize their group or population fitness? This requires extremely restrictive conditions, in which there is effectively no conflict within groups, such as in clonal populations of cells[63,74,75]. The cells that make up complex multicellular organisms, such as humans, fit this criterion, but populations or communities of microorganisms might not. Consequently, thinking about adaptations such as division of labour at the group or population level is not formally justified and can lead to errors with microorganisms.

As mentioned above, the expression of *S.* Typhimurium *ttss-1* in a subpopulation seems to be an altruistically cooperative trait, and hence represents division of labour[23,30]. In agreement with this, studies have shown that when grown in a monoculture, a mutant *S.* Typhimurium lineage that does not express *ttss-1* is avirulent and unable to spread within hosts (FIG. 1D). By contrast, when grown in a mixed culture with a ttss-1-expressing wild-type strain, the mutant lineage is at an advantage and expands (FIG. 1D). Similar data, showing that more cooperative strains can be exploited by less cooperative strains, have been reported with the fruiting bodies of *D. discoideum* and *M. xanthus*, and the tubular mitochondrial morphology in *C. gattii*[9,24,25].

It is necessary to test whether a trait is cooperative in the environmental conditions under which that trait evolved, or as near to it as possible[8]. The costs and benefits of traits vary under different environmental conditions, and wrong conclusions can be made if experiments are carried out in inappropriate environments[33]. For example, if an experiment is carried out under conditions in which a trait was not required, then the cooperative benefit to other cells would not be observed[31]. Determining whether division is cooperative can be harder when traits are more complex and/or when labour is divided into more types. For example, in *B. subtilis*, two cell types are required to facilitate migration (one task): cells that produce surfactin, a surfactant that decreases water surface tension, and cells that produce an extracellular polysaccharide matrix that glues cells together[34].

Why divide labour?

Why would natural selection favour a division of labour, with different individuals carrying out different tasks, rather than just have each cell carry out all of the tasks? Considering a simple case with two tasks, A and B, in which investment into these two activities must be traded off against each other, because time and energy spent on task A cannot be spent on task B. For example, task A might be reproduction and task B might be the secretion of a factor that causes an inflammatory response in the host, as is the case for *S.* Typhimurium[22,23]. A division of labour can be favoured when two conditions are met: there is an efficiency benefit from having different individuals carry out different tasks (specialization); the reproductive interests of the different individuals are aligned, such that cooperation is favoured.

Efficiency benefits. Division of labour requires an efficiency benefit from different individuals specializing in different tasks[7,10,35–39]. This means that when a cell puts a large effort into a task, it obtains a larger return per unit invested. Assuming linear costs per unit invested into a task, this requires that the slope of the relationship between the proportion of resources that a cell allocates to a task and the fitness return is accelerating[7,10,35,40] (FIG. 2a). An accelerating slope could arise if a task becomes more efficient as more effort is put into it, or if carrying out one of the tasks affects the ability to carry out the other task (they are best carried out in different locations). By contrast, if the fitness returns from a task are decelerating, with cells becoming less efficient at carrying out their task, natural selection would favour that all cells carry out both tasks (FIG. 2a; see Supplementary information S4 (box)).

A major problem in the study of division of labour is the lack of experimental data showing that the fitness return is accelerating[41]. To date, arguments for efficiency benefits have relied on indirect extrapolations, rather than direct experimental tests. For example, in some species of cyanobacteria, cells are divided into cells that photosynthesize and cells that convert nitrogen gas into ammonia (heterocysts)[16]. It has been argued that this division is favoured because nitrogenase, the nitrogen-converting enzyme, is rapidly degraded in the presence of oxygen, which is produced during photosynthesis[42]. A direct experimental test of this hypothesis would require the manipulation of the extent of the division of nitrogen fixation and photosynthesis between cells. Such a manipulation would enable a direct measurement of how the amount of nitrogen fixed varied with the amount of effort that was put into nitrogen fixation versus photosynthesis.

There are several other factors that could influence selection for division. For example, the relative returns from different tasks could vary depending on the physiological condition or size of the cell[43]. In some volvocine green algae, such as *Volvox carteri*, multicellular groups are composed of large germ cells that reproduce and smaller somatic cells that beat their flagella to keep the colony afloat[44,45]. This division seems to be favoured because large

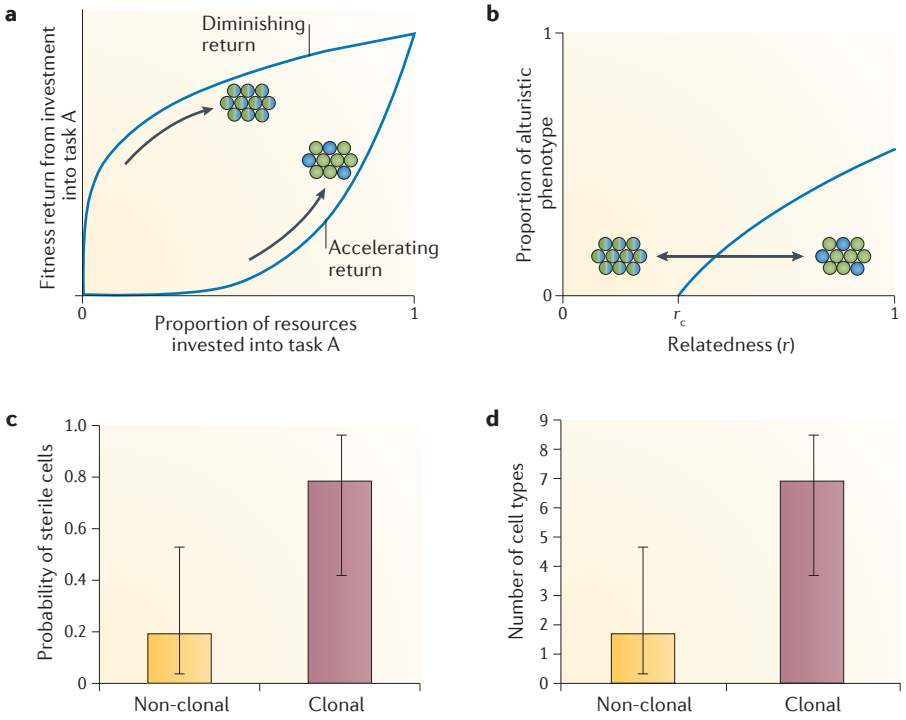


Figure 2 | **Why divide labour? a** | The relationship between the proportion of resources that a cell invests into task A and the fitness return from that task. We assume that a cell invests a proportion of resources (*X*) into task A, and the remaining proportion (1-*X*) into task B. Division of labour can be favoured when the returns from investment are accelerating. Cells can either exhibit division of labour, developing into a mixture of the altruistic helping phenotype (blue cells) and the reproducing phenotype (green cells), or exhibit no division of labour, developing into cells that carry out both the helping phenotype and reproduction (blue and green mixed-colour cells). **b** | The hypothetical relationship between the proportion (or likelihood) of individuals specializing in task A, and the relatedness (*r*) between interacting individuals in a social group. Division of labour is only favoured above a threshold value of relatedness (r_c). **c**,**d** | Among the kingdoms of life, a higher relatedness in multicellular groups correlates with a higher likelihood of sterile cells (part **c**), and more cell types (part **d**). The relatedness comparison is between groups that form clonally ($r=1$) and groups that form non-clonally ($r<1$). Part **c** and part **d** are adapted from REF. 53.

cells are more efficient at reproducing, which involves growth to a large cell size and then division, and small cells are better at both keeping the colony afloat and promoting the diffusion of nutrients across the colony wall[36,46,47]. Another possibility is that cells in poor condition, such as smaller sized or starved cells, could preferentially become altruistic helper cells rather than reproduce, if they have less to lose by not reproducing, compared with other cells in better condition.

A major research aim is to explain why some species divide labour whereas other similar species do not? For example, it seems reasonable that the interaction between nitrogen fixation and photosynthesis could lead to the accelerating fitness return in FIG. 2a, and hence lead to division of labour in species of cyanobacteria such as *Anabaena cylindrica*. However, this raises the question of how we explain other cyanobacteria such as *Trichodesmium erythraeum*[16,42], in which this division of labour has not been favoured.

Alignment of interests. Division of labour requires that the fitness interests of different individuals are aligned. If not, the interaction between these individuals could be destabilized by selfish cheats, which can exploit the cooperative nature of division of labour. Examples of such cheats would be a strain of *S.* Typhimurium that produced more cells that remain in the gut lumen and less cells that migrate to the gut tissue to express *ttss-1*, or a lineage of *D. discoideum* that invested more into spore cells and less into stalk cells[23,24,48].

One way for the interests of different individuals to be aligned is if they are genetically related[19]. This idea, often termed kin selection, proposes that by helping a relative to reproduce, an individual is still indirectly passing on copies of its own genes to the next generation (BOX 2). Consequently, related cells might be favoured to work together by dividing tasks to increase their genetic contribution to the next generation. Hamilton's rule[19] (BOX 2) predicts that altruistic traits (such as invading the gut tissue and expressing *ttss-1* in *S.* Typhimurium) will be favoured when both the relatedness between cells (*r*) and the efficiency benefits of cooperation (*B*/*C*) are sufficiently high (BOX 2). This illustrates that although division of labour is more likely with a higher relatedness, and that many examples of division are clonal ($r=1$), division of labour is also possible in non-clonal ($r<1$) groups.

Four lines of evidence support the hypothesis that relatedness is important in favouring division of labour within species. First, anecdotally, many examples of division of labour occur in groups that are clonal ($r=1$) or that are close to clonal. For example, cyanobacteria differentiate in clonal filaments[16], and the average relatedness in fruiting bodies of the slime mould *D. discoideum* is 0.98 (REF. 49). Second, some species that divide labour exhibit kin discrimination during group formation, which increases the relatedness within social groups. For example, individuals of *Dictyostelium purpureum* preferentially form fruiting bodies with clone mates[50]. Third, the maintenance of cultures with an artificially low relatedness led to the loss of the ability to form fruiting bodies in both *D. discoideum* and *M. xanthus*[51], and a lower investment into somatic functions in the fungus *Neurospora crassa*[52]. Fourth, comparing across species, species with clonal group formation have greater division of labour, with both a higher likelihood of sterile cells and more cell types than species in which group formation is non-clonal[53] (FIG. 2c).

What kind of division?

Having established whether division of labour is favoured, more subtle questions arise. For example, what proportion of individuals should carry out the different tasks? Between how many different cell types will labour be divided? And which mechanisms are expected to give rise to division of labour?

What proportion of individuals should carry out a task? Within the context of the theoretical example, in which individuals carry out either task A or task B, we can ask what the evolutionarily stable strategy[54] (ESS)

fraction of individuals is that should carry out task A and task B[22,35,42]. The concept of ESS is often used in evolutionary biology to denote the strategy or behaviour that would be selected over evolutionary time[55]. Put formally, it is the strategy which, if adopted by everyone in the population, cannot be replaced by any alternate strategy[54].

The ESS depends on the shape of the fitness-return curves (FIG. 2a) and the relatedness (*r*) between interacting cells. One prediction is that, in clonal populations, the fitness of individuals will peak at the ESS fraction of individuals that carry out task A and task B, and then decreases when individuals that carry out either task A or task B become more common[22]. For example, *S.* Typhimurium cells express enough *ttss-1* to trigger an inflammatory response, but any more than that is a waste[22]. Consistent with this, strains with either a lower or a higher proportion of mutant cells that express *ttss-1* showed a reduced fitness[23].

Theory could be developed for specific cases to help explain both what the ESS is for certain situations and in what way the ESS should vary across populations or species. For example, in the volvocine green algae, the ratio of soma cells to germ cells increases with colony size[44]. It has been argued that this represents the correlation between the shape of the fitness curve (FIG. 2a) and the changing group size. Specifically, as colony size increases, it becomes harder to keep the colony afloat and to transport nutrients, and therefore the ESS fraction of soma cells increases[36,46].

Other questions include why within species does the proportion of stalk cells in *D. discoideum* vary so markedly between samples taken from the same location[48,49]? And why does the proportion of stalk cells vary among closely related *Dictyostelium* species[56]. Moreover, among more distantly related species, why does the proportion of the altruistic reproductive phenotype vary from approximately 20% of *D. discoideum* cells that become stalk cells, to more than 99% of *Volvox* spp. cells that become soma cells[24,57]? ESS theory provides a tool for finding answers to these questions.

The ESS ratio of different phenotypes is also likely to vary with the relatedness between interacting individuals. However, there is a lack of both theory and empirical work that examines how variation in relatedness would influence the ESS ratio of phenotypes[22,53]. Previous theoretical analyses have focused on the extreme case of clonal populations (r=1). As relatedness decreases, it will lead to a decrease in the kin selected benefit from helping others[19]. Thus, we suspect that a general prediction is that a lower relatedness will lead to a lower proportion of individuals that express altruistic phenotypes (FIG. 2b). Comparing between species, the percentage of sterile cells is twice as high in species with clonal groups, but this pattern is not statistically significant[53]. However, there were only data available for a small number of phylogenetically independent comparisons, and thus this test had low statistical power, emphasizing the need to obtain data from a wider range of taxa.

How many types? Labour is sometimes divided into more than two phenotypes. For example, in cyanobacteria the number of cell types varies among species, up to at least four types[16]. These cell types include photosynthetic cells, nitrogen-fixing heterocysts, resting cells that are able to withstand environmental stress (akinetes) and motile dispersing filaments of cells (hormogonia).

We lack a formal theoretical framework to explain variation in the number of cell types. Various factors are likely to be important, including ecological conditions, molecular mechanisms, relatedness within groups and group size. Consistent with these possibilities, species that form clonal groups (r=1) have more cell types than species in which groups are not clonal ($r<1$) (FIG. 2d), and the number of cell types is positively correlated with group size[53,58]. A caveat here is that not all cell types represent division of labour, as phenotypic variation can arise for other reasons, such as bet hedging[2,3] (see above).

Which way to divide? Given that there are many ways to produce variable phenotypes within a species (BOX 1), should we expect one mechanism to dominate or different mechanisms to mediate phenotypic variation in different species? And if mechanisms vary across species, does this represent adaptive variation, with different mechanisms being better suited to different situations, or is phenotypic variation just the result of noise created by historical artefacts? There has been no theoretical or empirical work that has addressed such questions, and we therefore make several tentative suggestions.

We suspect that it would require restrictive conditions to maintain division of labour within species that are mediated by genetic differences. For example, most interactions in division of labour occur in clonal lineages, in which there will be insufficient genetic differences. Furthermore, when several genetic lineages interact, this can reduce selection for cooperation[59], and so although phenotypic variation can be maintained, it could be hard for such interactions to constitute a cooperative division of labour. For example, the coexistence of producing and non-producing lineages, that have traits such as invertase production in *Saccharomyces cerevisiae* or siderophore production in *P. aeruginosa*, seems to represent the coexistence of cooperators and cheats, and not cooperative division of labour[60,61]. Cooperation between different genotypes (or species) presents the problem of how to keep them together over evolutionary time (Supplementary information S3 (box)). Similar arguments would apply when considering epigenetic mechanisms[62].

By contrast, phenotypic noise seems to be a robust way to produce division of labour. Noise can produce one or more phenotypes from a single genotype. The ratio of these phenotypes could be selected for based on the underlying gene network to produce ratios of different phenotypes that vary according to the ESS in the local environment[2]. However, a limitation is that phenotypic noise could work less well in small social groups, as stochasticity would lead to a chance that there is none, or almost none, of a certain phenotype in a social group, which could result in a large fitness cost associated with maintaining phenotypic variation (FIG. 3). Consequently, we predict that phenotypic noise will more likely be the mechanism that is used when the social group is very large, which is exemplified by the expression of the *ttss-1* system in *Salmonella* spp. cells[22,23].

Coordination between cells by, for example, cell signalling, provides a possible solution to the problem of stochasticity in small social groups. If cells interact and coordinate phenotypes at a local level, then this can ensure a precise and appropriate ratio of different phenotypes, even in small social groups, as occurs in cyanobacterial filaments[16] (FIG. 3). Given this advantage of signalling, why is it not used more frequently to control division of labour? It is possible that this strategy could be costly, or that it would be ineffective in certain environments — for example, when diffusion rates are high. In such cases, and when the problem of stochasticity is less important, phenotypic noise could provide a more efficient mechanism to divide labour.

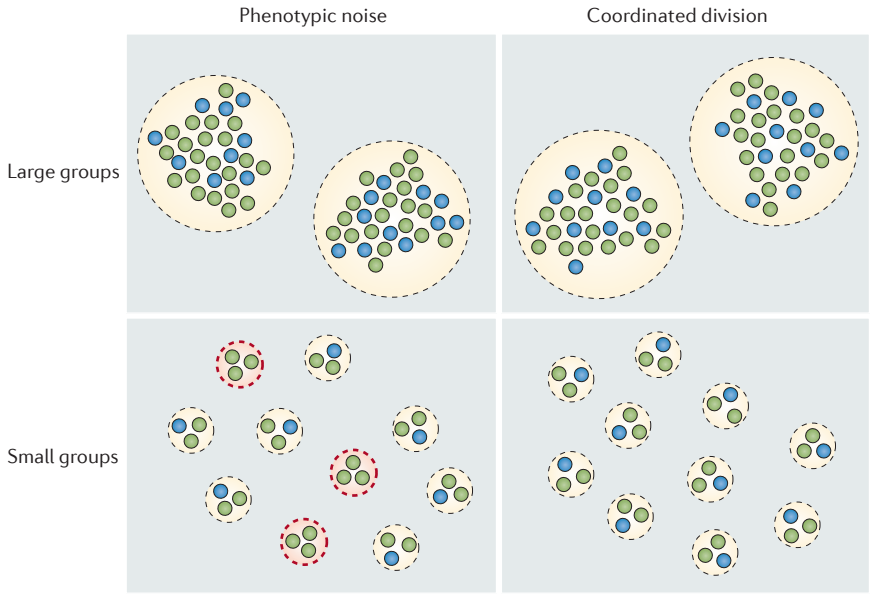


Figure 3 | **How to divide?** Considering a population that comprises altruistic cells (blue) and reproductive cells (green). Determination of phenotype occurs through phenotypic noise or through a mechanism, such as cell signalling, that coordinates division. The number of cells that interact in a social group can be large or small. In large groups, both phenotypic noise and coordinated division lead to groups with the evolutionary stable strategy (ESS) number of altruists. Consequently, both mechanisms could be favoured to establish division of labour. If coordinated division is more costly; for example, because it is metabolically costly to produce a signal, then phenotypic noise will be the favoured mechanism. In small social groups, owing to the stochastic nature of phenotypic noise, there is a chance that the group contains no altruists, and this group will consequently have poor fitness (these groups are circled in red). By contrast, coordinated division provides a mechanism to ensure that all groups contain altruists. Consequently, phenotypic noise is less likely to be favoured as a mechanism to divide labour in species in which the number of interacting cells is small.

Outlook

In this Opinion article, we have provided a definition of division of labour and discussed its implications. Is our definition useful? We have taken an evolutionary approach, focused on how individuals are adapted to their environments, and emphasized that division involves the cooperation of individuals to provide an inclusive fitness benefit to the entire social group. An alternative approach, focused on outcome rather than evolutionary adaptation, would be to define division of labour more loosely, such as when phenotypic diversity enables more complex tasks to be carried out[27]. This alternative approach would include the examples that we have excluded, such as diversification and one phenotype exploiting another.

The advantage of our stricter definition is that it integrates cases in which the same problems arise, and in which there is the potential for unifying understanding. For example, our stricter definition has highlighted the importance of shared interests and nonlinear fitness returns in the evolution of division of labour (FIG. 2). By contrast, a looser definition would integrate traits that have evolved for very different reasons and hence obscure underlying similarities. For example, the evolution of exploitation, or cheats that do not produce iron chelating siderophores, does not require nonlinear fitness returns, and is less likely to be favoured when individuals have shared interests (cheating is favoured by a lower and not a higher *r* value). This illustrates the advantage of drawing a clear distinction between processes that arise for different reasons[63].

Phenotypic heterogeneity between cells could arise in two ways; either through persistent specialization, whereby some cells only carry out task A, and other cells only carry out task B; or through transient specialization, whereby the same cell switches between carrying out task A or task B at different times. In the social insect literature, it has been suggested that persistent specialization is required for division of labour[64]. By contrast, by emphasizing the role of cooperation, our definition clarifies that both persistent and transient specialization can lead to division of labour.

Moreover, it has been suggested that the mutation of cooperators into non-cooperative cheats had a pivotal role in the evolution of the division between reproductive cells (germ cells) and helper cells (soma cells), and hence the evolution of complex multicellularity[65,66]. However, if we consider this hypothesis from an evolutionary perspective, both theory and empirical data contradict this notion. Theory suggests that selection would favour cheats that exploit cooperators and cooperators that are less likely to be exploited, which would lead to selection in the opposite direction than towards multicellularity[8,11]. Empirical data have shown that dividing labour between germ cells and soma cells occurs more often in species that have clonal group formation, in which there is no selection for cheating[53] (FIG. 2c).

Based on the several examples that have been described in recent years, these are exciting times for the study of division of labour in microorganisms. However, much more theoretical and empirical data are required. In many cases, it even remains to be shown whether phenotypic variation really represents division of labour. In cases in which division of labour can be established, further questions arise (see Supplementary information S5, S6, S7, S8, S9 (boxes)). Why is division favoured? How many different phenotypes exist, and what proportion of each phenotype? What mechanisms drive phenotypic diversity and why? Can we explain variation among species, as well as specific cases? Can we apply the same concepts to explain division of labour between species? By answering these questions, we can unify our understanding of division of labour, not only with regards to mechanistically different microbial examples, but also for other taxa, including animal societies.

Stuart A. West and Guy A. Cooper are at the Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.

Correspondence to S.A.W.
Stuart.West@zoo.ox.ac.uk

doi:10.1038/nrmicro.2016.111
Published online 19 Sep 2016

1. Lewis, K. Persister cells, dormancy and infectious disease. *Nat. Rev. Microbiol.* **5**, 48–56 (2006).
2. Veening, J.-W., Smits, W. K. & Kuipers, O. P. Bistability, epigenetics, and bet-hedging in bacteria. *Annu. Rev. Microbiol.* **62**, 193–210 (2008).
3. Ackermann, M. A functional perspective on phenotypic heterogeneity in microorganisms. *Nat. Rev. Microbiol.* **13**, 497–508 (2015).
4. Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. Bacterial persistence as a phenotypic switch. *Science* **305**, 1622–1625 (2004).
5. Veening, J.-W. *et al.* Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Mol. Syst. Biol.* **4**, 1–15 (2008).
6. Claessen, D., Rozen, D. E., Kuipers, O. P., Søgaard-Andersen, L. & van Wezel, G. P. Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies. *Nat. Rev. Microbiol.* **12**, 115–124 (2014).

7. van Gestel, J., Vlamakis, H. & Kolter, R. in *Microbial Biofilms* 2nd edn (eds Ghannoum, M., Parsek, M., Whiteley, M. & Mukherjee, P.) 67–97 (ASM press, 2015).
8. Ghoul, M., Griffin, A. S. & West, S. A. Toward an evolutionary definition of cheating. *Evolution* **68**, 318–331 (2014).
9. Voelz, K. *et al.* 'Division of labour' in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak. *Nat. Commun.* **5**, 5194 (2014).
10. Maynard Smith, J. & Szathmáry, E. *The Major Transitions in Evolution* (Oxford Univ. Press, 1998).
11. Bourke, A. F. G. *Principles of Social Evolution* (Oxford Univ. Press, 2011).
12. West, S. A., Fisher, R. M., Gardner, A. & Kiers, E. T. Major evolutionary transitions in individuality. *Proc. Natl Acad. Sci. USA* **112**, 10112–10119 (2015).
13. Crespi, B. J. & Yanega, D. The definition of eusociality. *Behav. Ecol.* **6**, 109–115 (1995).
14. West, S. A., Griffin, A. S. & Gardner, A. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Biol.* **20**, 415–432 (2007).
15. Tinbergen, N. On aims and methods of Ethology. *Zeitschrift Tierpsychol.* **20**, 410–433 (1963).
16. Flores, E. & Herrero, A. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat. Rev. Microbiol.* **8**, 39–50 (2009).
17. Davidson, C. J. & Surette, M. G. Individuality in bacteria. *Annu. Rev. Genet.* **42**, 253–268 (2008).
18. West, S. A., Griffin, A. S., Gardner, A. & Diggle, S. P. Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* **4**, 597–607 (2006).
19. Hamilton, W. D. The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**, 1–16 (1964).
20. Grafen, A. Optimization of inclusive fitness. *J. Theor. Biol.* **238**, 541–563 (2006).
21. West, S. A. & Gardner, A. Adaptation and inclusive fitness review. *Curr. Biol.* **23**, R577–R584 (2013).
22. Ackermann, M. *et al.* Self-destructive cooperation mediated by phenotypic noise. *Nature* **454**, 987–990 (2008).
23. Diard, M. *et al.* Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature* **494**, 353–356 (2013).
24. Strassmann, J. E., Zhu, Y. & Queller, D. C. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408**, 965–967 (2000).
25. Velicer, G. J., Kroos, L. & Lenski, R. E. Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature* **404**, 598–601 (2000).
26. Rainey, P. B. & Travisano, M. Adaptive radiation in a heterogeneous environment. *Nature* **394**, 69–72 (1998).
27. Kim, W., Levy, S. B. & Foster, K. R. Rapid radiation in bacteria leads to a division of labor. *Nat. Commun.* **7**, 10508 (2016).
28. Griffin, A. S., West, S. A. & Buckling, A. Cooperation and competition in pathogenic bacteria. *Nature* **430**, 1024–1027 (2004).
29. Jiricny, N. *et al.* Fitness correlates with the extent of cheating in a bacterium. *J. Evol. Biol.* **23**, 738–747 (2010).
30. Andersen, S. B., Marvig, R. L., Molin, S., Krogh Johansen, H. & Griffin, A. S. Long-term social dynamics drive loss of function in pathogenic bacteria. *Proc. Natl Acad. Sci. USA* **112**, 10756–10761 (2015).
31. Diggle, S. P., West, S. A., Griffin, A. S. & Campbell, G. S. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**, 411–414 (2007).
32. West, S. A., Winzer, K., Gardner, A. & Diggle, S. P. Quorum sensing and the confusion about diffusion. *Trends Microbiol.* **20**, 586–594 (2012).
33. Ghoul, M., West, S. A., Diggle, S. P. & Griffin, A. S. An experimental test of whether cheating is context dependent. *J. Evol. Biol.* **27**, 551–556 (2014).
34. van Gestel, J., Vlamakis, H. & Kolter, R. From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol.* **13**, e1002141 (2015).
35. Michod, R. E. Evolution of individuality during the transition from unicellular to multicellular life. *Proc. Natl Acad. Sci. USA* **104**, 8613–8618 (2007).
36. Michod, R. E., Viossat, Y., Solari, C. A. & Hurand, M. Life-history evolution and the origin of multicellularity. *J. Theor. Biol.* **239**, 257–272 (2006).
37. Ispolatov, I., Ackermann, M. & Doebeli, M. Division of labour and the evolution of multicellularity. *Proc. Biol. Sci.* **279**, 1768–1776 (2012).
38. Gavrilets, S. Rapid transition towards the division of labor via evolution of developmental plasticity. *PLoS Comput. Biol.* **6**, e1000805 (2010).
39. Oster, G. F. & Wilson, E. O. *Caste and Ecology in the Social Insects* (Princeton Univ. Press, 1978).
40. Charnov, E. L. *The Theory of Sex Allocation* (Princeton Univ. Press, 1982).
41. Koufopanou, V. & Bell, G. Soma and germ: an experimental approach using *Volvox*. *Proc. R. Soc. B: Biol. Sci.* **254**, 107–113 (1993).
42. Rossetti, V. *et al.* The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J. Theor. Biol.* **262**, 23–34 (2010).
43. Wilson, E. O. Ergonomics of caste in social insects. *Am. Nat.* **102**, 41–66 (1968).
44. Koufopanou, V. The evolution of soma in the Volvocales. *Am. Nat.* **143**, 907–931 (1994).
45. Herron, M. D., Hackett, J. D., Aylward, F. O. & Michod, R. E. Triassic origin and early radiation of multicellular volvocine algae. *Proc. Natl Acad. Sci. USA* **106**, 3254–3258 (2009).
46. Solari, C. A., Kessler, J. O. & Michod, R. E. A. Hydrodynamics approach to the evolution of multicellularity: flagellar motility and germ–soma differentiation in volvocalean green algae. *Am. Nat.* **167**, 537–554 (2006).
47. Solari, C. A., Ganguly, S., Kessler, J. O., Michod, R. E. & Goldstein, R. E. Multicellularity and the functional interdependence of motility and molecular transport. *Proc. Natl Acad. Sci. USA* **103**, 1353–1358 (2006).
48. Wolf, J. B. *et al.* Fitness trade-offs result in the illusion of social success. *Curr. Biol.* **25**, 1086–1090 (2015).
49. Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E. & Queller, D. C. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl Acad. Sci. USA* **104**, 8913–8917 (2007).
50. Mehdiabadi, N. J. *et al.* Kin preference in a social microbe. *Nature* **442**, 881–882 (2006).
51. Kuzdzal-Fick, J. J., Queller, D. C., Fox, S. A. & Strassmann, J. E. High relatedness is necessary and sufficient to maintain multicellularity in *Dictyostelium*. *Science* **334**, 1548–1551 (2011).
52. Bastiaans, E., Debets, A. J. M. & Aanen, D. K. Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. *Nat. Commun.* **7**, 1–10 (2016).
53. Fisher, R. M., Cornwallis, C. K. & West, S. A. Group formation, relatedness, and the evolution of multicellularity. *Curr. Biol.* **23**, 1120–1125 (2013).
54. Maynard Smith, J. & Price, G. R. The logic of animal conflict. *Nature* **246**, 15–18 (1973).
55. Dawkins, R. *The Selfish Gene* (Oxford Univ. Press, 1976).
56. Bonner, J. T. *Cellular Slime Molds* (Princeton Univ. Press, 1967).
57. Shelton, D. E., Desnitskiy, A. G. & Michod, R. E. Distributions of reproductive and somatic cell numbers in diverse *Volvox* (Chlorophyta) species. *Evol. Ecol. Res.* **14**, 707–727 (2012).
58. Bell, G. & Mooers, A. O. Size and complexity among multicellular organisms. *Biol. J. Linnean Soc.* **60**, 345–363 (1997).
59. Frank, S. A. Host–symbiont conflict over the mixing of symbiotic lineages. *Proc. Biol. Sci.* **263**, 339–344 (1996).
60. Gore, J., Youk, H. & van Oudenaarden, A. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**, 253–256 (2009).
61. Ross-Gillespie, A., Gardner, A., West, S. A. & Griffin, A. S. Frequency dependence and cooperation: theory and a test with bacteria. *Am. Nat.* **170**, 331–342 (2007).
62. Haig, D. Weismann rules! OK? Epigenetics and the Lamarckian temptation. *Biol. Philos.* **22**, 415–428 (2006).
63. Maynard Smith, J. Group selection. *Q. Rev. Biol.* **51**, 277–283 (1976).
64. Gordon, D. M. From division of labor to the collective behavior of social insects. *Behav. Ecol. Sociobiol.* **70**, 1101–1108 (2015).
65. Rainey, P. B. & Kerr, B. Cheats as first propagules: a new hypothesis for the evolution of individuality during the transition from single cells to multicellularity. *Bioessays* **32**, 872–880 (2010).
66. Hammerschmidt, K., Rose, C. J., Kerr, B. & Rainey, P. B. Life cycles, fitness decoupling and the evolution of multicellularity. *Nature* **515**, 75–79 (2015).
67. Veening, J.-W. *et al.* Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc. Natl Acad. Sci. USA* **105**, 4393–4398 (2008).
68. Kirk, D. L. Asymmetric division, cell size and germ–soma specification in *Volvox*. *Semin. Dev. Biol.* **6**, 369–379 (1995).
69. Schmitt, R. Differentiation of germinal and somatic cells in *Volvox carteri*. *Curr. Opin. Microbiol.* **6**, 608–613 (2003).
70. Nedelcu, A. M. & Michod, R. E. The evolutionary origin of an altruistic gene. *Mol. Biol. Evol.* **23**, 1460–1464 (2006).
71. Hanschen, E. R., Ferris, P. J. & Michod, R. E. Early evolution of the genetic basis for soma in the Volvocaceae. *Evolution* **68**, 2014–2025 (2014).
72. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, 1930).
73. Grafen, A. The formal Darwinism project: a mid-term report. *J. Evol. Biol.* **20**, 1243–1254 (2007).
74. Leigh, E. G. When does the good of the group override the advantage of the individual? *Proc. Natl Acad. Sci. USA* **80**, 2985–2989 (1983).
75. Gardner, A. & Grafen, A. Capturing the superorganism: a formal theory of group adaptation. *J. Evol. Biol.* **22**, 659–671 (2009).
76. Shelton, D. E. & Michod, R. E. Philosophical foundations for the hierarchy of life. *J. Evol. Biol.* **25**, 391 (2010).
77. Sturm, A. *et al.* A. The cost of virulence: retarded growth of *Salmonella* Typhimurium cells expressing type III secretion system 1. *PLoS Pathog.* **7**, e1002143 (2011).

Acknowledgements

The authors thank K. Boomsma, A. P. Escudero, K. Foster, A. Gardner, M. Ghoul, J. Gore, A. Griffin, R. May, J. Strassmann, D. Unterweger and J. van Gestel for very useful discussions. The authors also thank M. Ackermann, R. May, R. Michod and J.-W. Veening for kindly providing images. G.A.C was funded by the Engineering and Physical Sciences Research Council (EPSRC).

Competing interests statement

The authors declare no competing interests.

SUPPLEMENTARY INFORMATION

See online article: S1 (box) | S2 (box) | S3 (box) | S4 (box) | S5 (box) | S6 (box) | S7 (box) | S8 (box) | S9 (box)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Supplementary materials 3. Division of labour and the evolution of extreme specialisation (published paper)

nature ecology & evolution

ARTICLES

https://doi.org/10.1038/s41559-018-0564-9

Division of labour and the evolution of extreme specialization

Guy A. Cooper* and Stuart A. West

Division of labour is a common feature of social groups, from biofilms to complex animal societies. However, we lack a theoretical framework that can explain why division of labour has evolved on certain branches of the tree of life but not others. Here, we model the division of labour over a cooperative behaviour, considering both when it should evolve and the extent to which the different types should become specialized. We found that: (1) division of labour is usually—but not always—favoured by high efficiency benefits to specialization and low within-group conflict; and (2) natural selection favours extreme specialization, where some individuals are completely dependent on the helping behaviour of others. We make a number of predictions, several of which are supported by the existing empirical data, from microbes and animals, while others suggest novel directions for empirical work. More generally, we show how division of labour can lead to mutual dependence between different individuals and hence drive major evolutionary transitions, such as those to multicellularity and eusociality.

Division of labour is a defining feature of complexity at all levels of biological organization[1–5]. If individuals specialize to perform certain tasks, more complex social groups can evolve. In the extreme, if the different individuals become dependent on the tasks performed by others, then a new 'higher level' individual may emerge. Examples include genes with different functions in a genome, cells that form distinct tissues in an animal and castes that carry out different tasks in social insect societies. Consequently, to understand why complex life has evolved, we must understand the evolution of division of labour.

We lack theory that can explain why division of labour has evolved on some branches of the tree of life, but not others. Previous work has focused on clonal groups of cells and eusocial insects[6–15]. In both of these cases, it has usually been assumed that the fitness interests of individuals are perfectly aligned, and so the evolution of division of labour is favoured if it increases group fitness (Supplementary Tables 1 and 2). However, division of labour also arises in species such as bacteria, slime moulds and cooperatively breeding animals, where there can be appreciable conflict within groups and so cannot be assumed to be 'superorganisms'[2,16,17]. If there is conflict within groups, then division of labour would not be selected for just because it increases group fitness[18–20]. Furthermore, if division of labour plays a role in driving transitions such as those to multicellularity and eusociality, then we need to understand how it can first evolve from individual level selection[2,4,21].

Division of labour can take different forms (Fig. 1). In the simplest possible scenario, with only one cooperative behaviour, a division of labour consists of 'helpers' and 'reproductives' that may be specialized to varying degrees. The helper could be a fully specialized, sterile helper or a generalist that both helps and reproduces. Similarly, the more reproductive type could be a pure reproductive or a generalist that engages in some helping. This suggests four broad types of division of labour—from two different generalist types that help and reproduce at different rates, to the extreme case of a sterile helper paired with a pure reproductive (Fig. 1). However, most models assume that only a certain type of division of labour is possible, often with fully specialized sterile helpers[7,9,10,12,19] (Supplementary Tables 1 and 2). Therefore, these models cannot be used to explain variation in the form that division of labour takes.

We theoretically model how a number of factors could influence selection for division of labour and its various forms. We wish to find the conditions that would favour both the initial evolution of division of labour, and the evolution of extreme specialization, with individuals losing the ability to reproduce independently. We are interested in insights that could be applied across a range of different biological systems. Consequently, we construct a deliberately simple approximation, focusing on the trade-offs that we hypothesize are likely to be of general importance, rather than a complex model of a specific system[22].

Results

We consider an infinite population that is divided into social groups of fixed, finite sizes in which individuals engage in social interactions locally but offspring compete globally for niches in the next generation (island model). The genetic relatedness between individuals in a social group is given by R, which represents the relative probability that they are identical by descent (see Methods).

We allow individuals to perform a costly cooperative behaviour, which increases the survival or reproductive viability of social group members. Specifically, a fraction $1-\lambda$ of the benefit of cooperation goes to the focal individual and the remaining fraction λ is distributed to the other members of the social group (Fig. 2c). We allow for potential efficiency benefits from greater cooperation with the parameter α (Fig. 2b), which determines whether the benefits from increased cooperation are linear ($\alpha=1$), accelerating ($\alpha>1$) or decelerating ($\alpha<1$). We vary the extent to which the cooperative trait is essential for survival with the parameter e. If $e=1$, then cooperation is essential and individuals that reside in social groups with no cooperation have a fitness of zero. As e decreases, the trait is less essential and the cooperative behaviour becomes more of a luxury activity.

We allow for a division of labour into two phenotypes (Fig. 2a). At the start of their life cycles, individuals terminally adopt phenotype 1 with probability p and phenotype 2 with probability $1-p$. Phenotype 1 invests a fraction q_1 of its lifetime efforts in the

Department of Zoology, University of Oxford, Oxford, UK. *e-mail: guy.cooper@zoo.ox.ac.uk

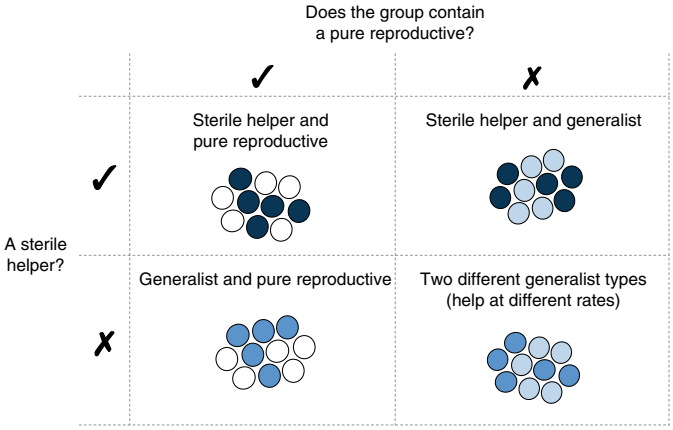


Fig. 1 | The different possible forms of division of labour. There are four broad forms of reproductive division of labour, each defined by the presence or absence of the two fully specialized phenotypes: pure reproductives (that is, germline cells or social insect queens) and sterile helpers (that is, somatic cells or worker castes). A sterile helper and pure reproductive division of labour (top left) is composed of both fully specialized phenotypes. The three other kinds of division of labour contain at least one generalist phenotype that invests in both tasks. In the strategy containing two different generalist phenotypes (bottom right), one of these phenotypes is cooperating at a higher level than the other.

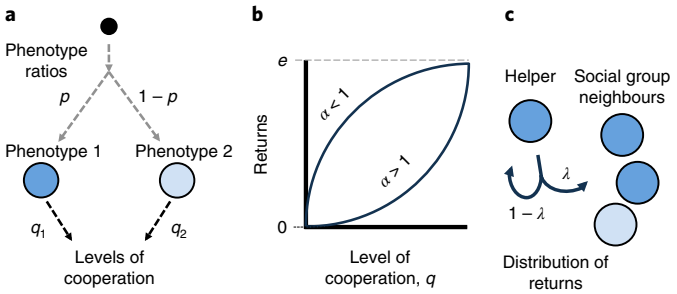


Fig. 2 | A division of labour model. We consider a cooperative trait that individuals may invest in at a private cost. **a**, The evolving traits. At the start of its life cycle, an undifferentiated individual (top, shaded) stochastically adopts one of two phenotypes (middle). Individuals become phenotype 1 with probability p and phenotype 2 otherwise. Each phenotype invests a fixed amount of lifetime effort (q_1 or q_2) into the cooperative trait (bottom). When the two phenotypes differ, we assume that phenotype 1 invests more into cooperation ($q_1 > q_2$). The traits p, q_1 and q_2 are the characteristics that are allowed to evolve in the model. **b**, The benefits of cooperation. We allow for accelerating ($\alpha > 1$) or diminishing ($\alpha < 1$) returns to increased investment in cooperation. Each individual has a baseline benefit $1-e$ regardless of the social environment. If $e = 1$, the trait is essential. Otherwise ($e < 1$), it is non-essential. The maximal return that can be attained via cooperation is then given by e (trait essentiality). **c**, The trait sociality. A proportion λ of the returns from personal investment in cooperation will benefit social group neighbours equally (others only; focal helper excluded). The remaining $1-\lambda$ benefits the focal helper alone. An additional parameter, R, quantifies the degree of relatedness within social groups of the population.

cooperative trait and the remaining fraction $1-q_1$ is allocated towards personal survival or reproduction. In contrast, phenotype 2 invests q_2 in the cooperative trait. When the two phenotypes differ, we will assume that $q_1 > q_2$, such that, without loss of generality, phenotype 1 is more cooperative. Consequently, we are allowing three independent traits to co-evolve in our model: the level of cooperation of each phenotype (q_1, q_2), and the relative ratio of the two phenotypes (p). In our analysis, we used equilibrium theory to determine the strategy that is expected to evolve in the long-term (see Methods and Supplementary Sections 1–3)[22,23]. The key predictions of our model are given in Table 1.

What types of division of labour are stable? Our model allows several possible strategies: uniform non-cooperation (no individuals help), uniform cooperation (all individuals are identical generalists that both help and reproduce) and four different types of division of labour (Fig. 1). The types of division of labour are defined by the presence or absence of the extreme possible phenotypes: sterile helpers ($q_1 = 1$) and pure reproductives ($q_2 = 0$; Fig. 1). We found that uniform non-cooperation, uniform cooperation and division of labour could all arise as long-term evolutionary strategies (Fig. 3).

We found that there was an evolutionary bias to more extreme forms of division of labour, where one of the phenotypes does all of the cooperation (Fig. 3). The two types of division of labour that could be favoured were those with a pure reproductive ($q_2 = 0$) paired with either a generalist helper ($0 < q_1 < 1$) or with a sterile helper ($q_1 = 1$). In contrast, we did not find a region of parameter space where either of the other two types of division of labour, in which both phenotypes engage in cooperation, could evolve (Fig. 3). Specifically, the combination of a generalist ($0 < q_2 < 1$) with either a more cooperative generalist ($q_2 < q_1 < 1$) or with a sterile helper ($q_1 = 1$) was never found to be stable. In Supplementary Section 4, we show that these results hold if we relax the assumption that cooperative costs are linear.

Why are intermediate forms of division of labour, where both phenotypes cooperate, not stable? We hypothesize that there may be an evolutionary feedback loop in which helper specialization drives reproductives to help less and reproductive specialization drives helpers to help more. To test this hypothesis, we developed dynamic, individual-based simulations as a proof of principle (Fig. 4 and Supplementary Section 5).

We held the level of cooperation in one phenotype fixed (q_1 or q_2) and allowed the other phenotype to evolve. We found that when phenotype 2 invested more resources into reproduction, phenotype 1 invested more resources into cooperation (lower q_2 drives higher q_1; Fig. 4a). In turn, when phenotype 1 invested more resources into cooperation, phenotype 2 was driven more rapidly to pure reproduction ($q_2 = 0$; Fig. 4b). More generally, the higher we fixed the level of cooperation of one phenotype, the higher the investment into reproduction of the other phenotype (Fig. 4c).

To examine how these effects feedback on to each other, we considered the consequences of allowing just one phenotype to evolve for some time and before allowing both phenotypes to evolve. We initially held fixed the level of cooperation of phenotype 2 ($0 < q_2$ fixed), which led to the other phenotype evolving to an intermediate level of cooperation ($q_1 < 1$; Fig. 4d). When we then allowed both phenotypes to evolve, they always drove each other to the specialist extremes of pure reproduction ($q_2 = 0$) and sterile helping ($q_1 = 1$; Fig. 4d).

The only intermediate form of division of labour that we find to be stable is the pairing of a generalist with a pure reproductive ($0 < q_1 < 1$; $q_2 = 0$). In the section 'Ecological benefits and further predictions', we discuss how one of the conditions required for division of labour to be favoured is that there are efficiency benefits to specialization ($\alpha > 1$). If division arises, we also found that the same condition ($\alpha > 1$) always favours the stability of pure reproduction ($q_2 = 0$). Consequently, whenever division of labour evolves, one phenotype will always be a pure reproductive ($q_2 = 0$). In contrast, efficiency benefits to specialization ($\alpha > 1$) are necessary but not sufficient for the stability of a sterile helper ($q_1 = 1$). The evolution of a sterile helper therefore requires more restrictive conditions then a

Table 1 | Model predictions and data for the evolution of division of labour

	Model predictions	Data
When is division of labour favoured?	1. (a) If the trait is non-essential ($e<1$), higher relatedness (higher R) favours division of labour. (b) If the trait is essential ($e=1$), there is no effect of the value of relatedness.	Clonal cell groups ($R=1$) are more likely to have a division of labour[24]. In animal groups, lower levels of promiscuity (higher R), leads to individuals being more likely to spend time as a helper in cooperative breeding vertebrates[33,34]. In all cases, distinction between essential and non-essential traits is not tested.
	2. (a) If relatedness, trait sociality and trait essentiality are high (higher R, λ and e), a higher efficiency benefit to specialization (higher α) favours division of labour. (b) Otherwise, uniform non-cooperation may be favoured.	Formal test needed. However, greater group size does correlate with division of labour in some systems and this may be due to altered efficiency benefits[6,26,30].
	3. Higher trait sociality (higher λ) favours division of labour.	–
	4. If relatedness and trait sociality are low and the efficiency benefits are high (low R and λ; high α), a higher trait essentiality (higher e) favours division of labour.	–
	5. Depending on how group size (N) influences factors such as the efficiency benefits to specialization (α), the extent to which the benefits of cooperation are shared (λ) or social group relatedness (R), a larger group may favour or disfavour division of labour.	Larger colony sizes have been found to favour division of labour in volvocine algae[6].
What kind of division is favoured?	6. The only forms of division that are favoured are those with a pure reproductive ($q_2=0$) paired with either a sterile helper ($q_1=1$) or a helper reproductive ($0<q_1<1$).	Formal test needed. Of the seven discussed examples of microbial division of labour, five are sterile helper and pure reproductive, one is generalist and pure reproductive and one is sterile helper and generalist division of labour[25–30,49].
When are sterile helpers favoured?	7. Higher relatedness (higher R) favours helper sterility.	Clonal cell groups ($R=1$) are more likely to have sterile cells[23]. Sterile helpers are disfavoured at low relatedness in both slime moulds and fungi[35,36]. In animal groups, eusociality has only evolved under conditions of strict lifetime monogamy (higher R)[3,32,37].
	8. (a) If relatedness, trait essentiality and trait sociality are high (high R, e and λ), higher efficiency benefits to specialization (higher α) favours helper sterility. (b) Otherwise, higher efficiency benefits (higher α) may favour uniform non-cooperation.	–
	9. Higher trait sociality (higher λ) favours helper sterility.	–
	10. If relatedness and trait sociality are low and the efficiency benefits are high (low R and λ; high α), higher trait essentiality (higher e) favours helper sterility.	–
What affects the proportion of helpers?	11. Higher relatedness (higher R) favours a higher proportion of helpers (higher p^*).	Clonal cell groups ($R=1$) have a higher proportion of helpers but study lacks phylogenetically independent comparisons and so is not statistically significant (more data needed)[24].
	12. (a) If the helpers are sterile ($q_1=1$), a higher efficiency benefits to specialization (higher α) favours a lower proportion of helpers (lower p^*). (b) Otherwise, there is no effect.	–
	13. (a) If helpers are sterile ($q_1=1$), higher trait sociality (higher λ) favours a higher proportion of helpers (higher p^*). (b) Otherwise, higher trait sociality (higher λ) favours a lower proportion of helpers (lower p^*).	–
	14. Higher trait essentiality (higher e) favours a higher proportion of helpers (higher p^*).	–

We present the key predictions of our model with respect to the conditions in which division of labour is favoured, what kind of division may be favoured, whether the extreme form of division with sterile helpers and pure reproductives is favoured and the factors that affect the proportion of helpers (if division is favoured.) We also specify whether the predictions have been previously tested empirically. The entry '–' indicates that an empirical test is needed.

pure reproductive and thus an intermediate division of labour composed of the former phenotype but not the latter would never occur ($q_1=1$; $q_2>0$).

Division of labour in nature. Our prediction that more extreme forms of division of labour should be observed corresponds to patterns observed in the natural world. Considering cell groups, the most common form of division appears to be between sterile helpers and pure reproductives[16,24]. One of the clearest examples is the germ–soma divide in multicellular animals. Similarly, in microorganisms such as bacteria, fungi, algae and slime moulds, there are numerous examples of a sterile helper paired with a pure reproductive[19,25–28]. In contrast, less extreme division of labour involving a generalist paired with either a pure reproductive or a sterile helper appears to be relatively rare, with a single example of each from bacteria and algae, respectively[29,30].

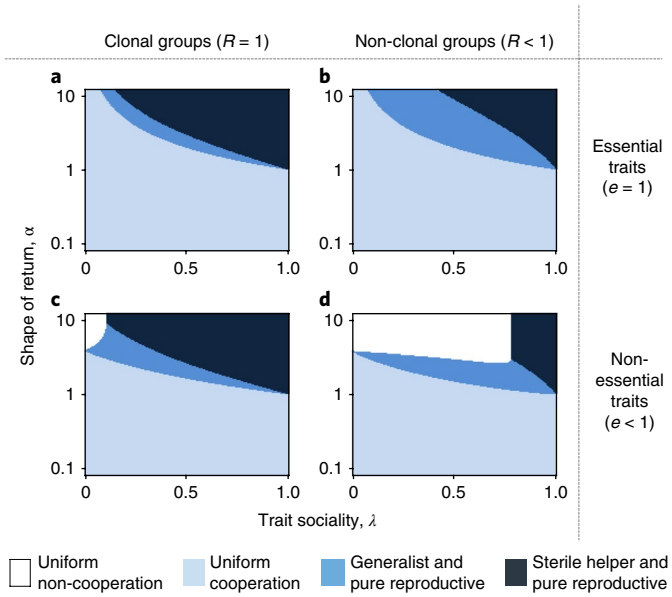


Fig. 3 | The evolution of division of labour. We examine how the various factors influence the strategy that is expected to evolve. Intermediate division of labour composed of generalists and pure reproductives is broadly favoured by increasing benefits to specialization, high trait sociality, high trait essentiality and high relatedness. If these factors are pushed to further extremes, then an extreme form of division of labour with sterile helpers and pure reproductives may be favoured. No other form of division of labour is observed to be stable. See Supplementary Sections 1–3 for more details. **a**, Higher trait essentiality and relatedness ($e = 1$; $R = 1$). **b**, Higher trait essentiality and lower relatedness ($e = 1$; $R = 1/7$). **c**, Lower trait essentiality and higher relatedness ($e = 9/10$; $R = 1$). **d**, Lower trait essentiality and relatedness ($e = 9/10$; $R = 1/7$).

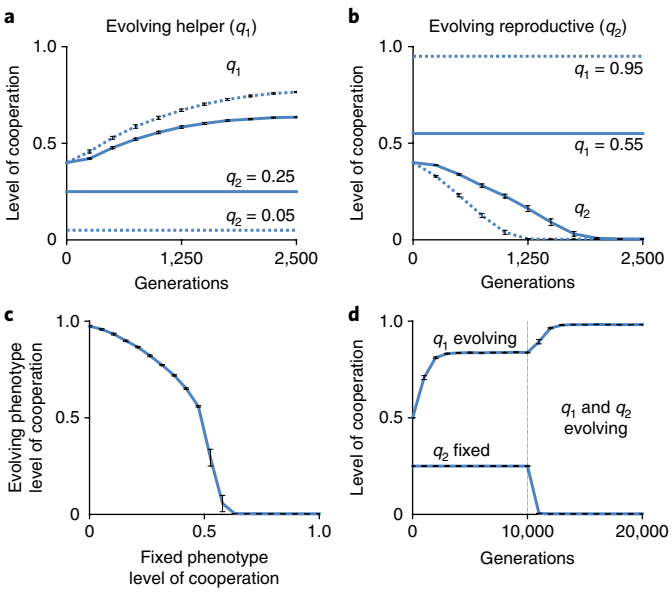


Fig. 4 | The evolution of extreme specialization. We hypothesized that there exists an evolutionary feedback loop whereby helper specialization drives reproductives to specialize further on reproduction and reproductive specialization drives helpers to help more. As a proof of principle, we tested this hypothesis with dynamic, individual-based simulations. **a**, We find that the level of cooperation of the helper phenotype, q_1, evolves to higher levels of cooperation when the reproductive phenotype, q_2, is more fully specialized (low q_2; dashed) than when it is less specialized (high q_2; solid). **b**, We find that the level of cooperation of the reproductive phenotype, q_2, evolves more quickly to pure reproduction ($q_2 = 0$) when the helper phenotype, q_1, is more fully specialized (high q_1; dashed) than when it is less specialized (low q_1; solid). **c**, If we hold one phenotype fixed, we find that the lower the level of cooperation of the fixed phenotype, the higher the level of cooperation of the evolving phenotype, and vice versa. **d**, If we hold reproductive specialization (q_2) fixed for 10,000 generations, then the level of helper cooperation (q_1) evolves stably to an intermediate value. If we then allow the level of cooperation of both phenotypes (q_1 and q_2) to evolve for another 10,000 generations, then both phenotypes are driven to their specialized extremes ($q_1 = 1$ and $q_2 = 0$.) All error bars are 95% confidence intervals over simulation repetitions. See Supplementary Section 5 for more details.

In animal groups, two forms of division of labour seem to be most common. First, in the social insects, the divide between queens and their workers is between pure reproductives and sterile or effectively sterile workers[2,3,7]. Second, in cooperative breeding vertebrates and invertebrates, the division is commonly associated with age—individuals help when young and breed when old[2,17]. This is akin to generalist and pure reproductive division of labour if only some individuals stay and help or, alternatively, uniform cooperation if all individuals do so. While our model captures the essence of why division of labour is favoured for these species, they also introduce a number of other factors, such as costs and benefits of cooperation varying with age, relatedness asymmetries and individuals who are 'failed breeders'[17,31]. However, as predicted by our model, there are no known instances of division of labour in animals between a sterile helper and a generalist (that engages both in breeding and in helping others breed).

Our examination of the pattern in nature requires two points of clarification. First, in all these cases, the appropriate comparison is one trait at a time. So, pure reproductives with respect to one trait may engage in other cooperative behaviours. For example, in the cyanobacterial division of labour, the cells that do not fix nitrogen are pure reproductives with respect to that trait, but can perform other cooperative traits, such as photosynthesis[25]. Second, there may be an observation bias towards discovering more extreme division of labour. Our prediction emphasizes the need for a quantitative survey of the types of division of labour in nature, rather than a reliance on just the systems that are being studied.

Our results do not categorically forbid the other two other types of division of labour in nature. Instead, our analysis offers a simple null model such that, if a form of division of labour not predicted by our model has evolved, then there must exist a complexity in the biological system not captured by our model and its assumptions. An example is provided by division of labour between sterile helpers and generalists in some volvocine algae lineages[30]. When these algae reproduce, the reproductive cells must grow to the size of offspring colonies before reproduction. As such, any reproduction comes with a large commitment of resources, leading to helper sterility providing a large discontinuous resource bonus not contained in our model[6].

Relatedness and division of labour. A standard assumption has been that a higher relatedness favours division of labour[2,16,24]. Indeed, many models of division of labour have assumed the extreme relatedness of clonality, or that it is group fitness that is being maximized[6,7,9,10,12]. In contrast, we found that relatedness (R) has no influence on whether division of labour is favoured for essential traits ($e = 1$) that are required for reproduction or survival (Fig. 3a,b). A higher relatedness has no influence because the fitness benefit of being a pure reproductive is then exactly cancelled by the indirect fitness cost of not helping relatives in the group.

However, for non-essential traits ($e<1$), a higher relatedness (higher R) does favour the evolution of division of labour (Fig. 3c,d). The main reason for this is that, as relatedness decreases, the indirect benefits of cooperation are reduced, and so uniform non-cooperation can outcompete cooperative division of labour[19]. Combining our trends, the overall prediction is that a higher relatedness (higher R) will favour division of labour for some traits (non-essential; $e<1$), but not for other traits (essential; $e=1$). The extent to which a trait is essential may change over evolutionary time—for example, a trait might start as relatively non-essential, and then become more essential as a group becomes more social, with more division of labour. In this case, relatedness could be more important for the initial evolution of division of labour than for its later maintenance. An empirical example is the subsequent loss of strict lifetime monogamy in some eusocial insects[32].

In the empirical data from multicellular groups, a higher relatedness is correlated with a greater likelihood of division of labour[24]. This is consistent with our model if the data are drawn only from non-essential traits, or a mix of essential and non-essential traits. In animal groups, a higher relatedness, due to lower levels of promiscuity, also leads to individuals being more likely to spend time as a helper in cooperative breeding vertebrates[33,34]. Our predictions suggest that it would be useful to further divide traits on the basis of how essential they are, and then test for how this interacts with relatedness.

Considering the different types of division of labour, our model predicts that a higher relatedness (higher R) favours more extreme division of labour regardless of whether the trait is essential or non-essential ($0<e\leq 1$; Fig. 3). In particular, a higher relatedness favours division between a sterile helper and pure reproductive ($q_1=1$, $q_2=0$) over division between a generalist and a pure reproductive ($0<q_1<1$, $q_2=0$).

Our predicted influence of relatedness is consistent with the empirical data for multicellular groups, where groups with a higher relatedness are more likely to have sterile helpers[24]. Experimental evolution studies have also found that the sterile helpers are disfavoured at relatively low relatedness, in both slime moulds and fungi[35,36]. In animal groups, the division between sterile helper and pure reproductive also appears to be favoured by a higher relatedness, with eusociality having only evolved in sexual species that have strict lifetime monogamy or asexual species that reproduce clonally[3,32,37].

In contrast to our predictions and the empirical data, some have argued that monogamy (higher R) may sometimes disfavour cooperation and division of labour[38,39]. However, subsequent work showed that these conclusions are based on restrictive assumptions. For example, in Nonacs's model, the best way for individuals to 'help' relatives is to disperse and reduce competition for resources rather than to stay and help kin[38,40]. Olejarz et al.'s results are an artefact of constraining the analysis to the invasion of unconditionally expressed worker sterility in colonies where only an intermediate proportion of sterile workers is optimal (Supplementary Section 7.5)[39,41].

Clonal groups and lifetime monogamy. While higher relatedness tends to favour division of labour, our model shows that maximal relatedness ($R=1$) is not required for division of labour to evolve, or even for the most extreme form of division between sterile helpers and pure reproductives ($q_1=1$, $q_2=0$; Fig. 3)[19]. Many previous models of division of labour have assumed maximal relatnedness ($R=1$), such that there is no conflict within groups, and analysed how division of labour can maximize group fitness[6,7,9,11,12]. We have shown that division of labour can still be favoured, even with relatively low relatedness ($R<1$) where there can be appreciable within-group conflict. This is consistent with Hamilton's rule, which showed how altruistic sterile helping can be favoured when $R<1$ (refs [18,19]).

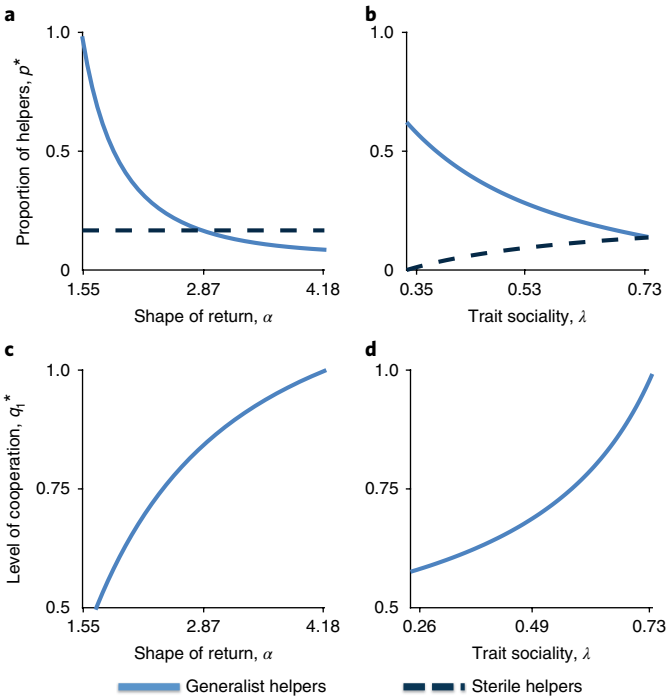


Fig. 5 | The proportion of helpers and the level of cooperation. Our model makes a number of predictions about what form division of labour should take, including the optimal proportion of helpers and their level of cooperation. We found that the way certain factors affect the proportion of helpers depends on the form of division of labour that is favoured. **a**, If the helpers are sterile ($q_1^*=1$), then an increase in the efficiency benefits of specialization (higher α) has no effect on the optimal proportion (p^*) of helpers ($q_1^*=1$). However, if the helpers are generalists ($0<q_1^*<1$), then a higher α decreases the optimal proportion of helpers (lower p^*). **b**, If the helpers are sterile ($q_1^*=1$), then an increase in the sociality of the trait (higher λ) increases the optimal proportion (higher p^*) of helpers ($q_1^*=1$). However, if the helpers are generalists ($0<q_1^*<1$), then a higher λ decreases the optimal proportion of helpers (lower p^*). These different predictions arise because, when there are generalists, the amount that they help (q_1^*) also changes. **c**, So for example, with a high efficiency benefit (higher α), we predict few generalists (lower p^*; **a**) but who help a lot (high q_1^*; **c**). In contrast, an increase in social group relatedness (higher R) or trait essentiality (higher e) leads to an increase in the optimal helper proportion (higher p^*) regardless of the form of division that is favoured ($0<q_1^*\leq 1$). **d**, For high trait sociality (higher λ), we predict fewer generalists (lower p^*; b) but who invest highly in cooperation (higher q_1^*; **d**). See Supplementary Section 7.4 for more details.

More generally, this emphasizes how division of labour can be favoured by kin selection at the level of the individual rather than simply by group efficiency maximization.

Our prediction that maximal relatedness is not necessary is supported by cases where division of labour with sterile and reproductive helpers has been observed in non-clonal multicellular groups[24,27]. In social insects, lifetime monogamy leads to a potential helper being equally related to their siblings and their own offspring, which is equivalent to $R=1$ in our asexual model[3,20,24,32]. Consequently, although eusociality has only evolved in species with lifetime monogamy or asexual reproduction, our theory shows that the initial evolution of division of labour, while favoured by maximal relatedness, does not require this condition in principle.

Ecological benefits and further predictions. Many previous models found that division of labour is favoured when there is an efficiency

benefit to specialization, with nonlinear returns to increased cooperation ($\alpha > 1$)[6,11,12,15]. In Supplementary Section 7.1, we show that an efficiency benefit to specialization ($\alpha > 1$) is necessary, but not sufficient for the evolution of division of labour (Fig. 3)[9,42]. Instead our model also makes a suite of predictions for how the efficiency benefits of increased cooperation interact with a number of other factors (Table 1 and Supplementary Sections 7.2 and 7.3). For example, division of labour is more likely to evolve if the benefits of cooperation are generously shared between individuals (high λ) and if the trait is very essential for survival (high e).

Our model also makes predictions about the factors that favour the most extreme form of division of labour, with sterile helpers and pure reproductives (high α, λ, e and R), and the factors that determine the optimal ratio of helpers to reproductives (p^*; Table 1 and Supplementary Section 7.4). These different factors can interact in unforeseen ways that qualitatively change predictions. For example, whether an increase in efficiency benefit of specialization (α) and trait sociality (λ) leads to higher, lower or has no influence on the optimal proportion of helpers (p^*) can depend on the type of division of labour that is favoured (Fig. 5).

Life history and population demography. As we are interested in patterns that hold across a range of different biological systems, we constructed a deliberately simple model, focusing on the factors that we believe are likely to be of broad importance (see Methods). For example, we purposefully left relatedness as an independent parameter ('open' model) and assumed that competition for breeding spots was global[22]. In some cases, for specific species, or groups of species, the way that the demographic processes generate relatedness patterns may be important for the evolution of division of labour. For these cases, our predictions may not hold and it could be useful to develop 'closed' models to examine how relatedness is determined by population demography and to make more targeted predictions[43]. We solve a closed model in Supplementary Section 6 and show that limited dispersal and overlapping generations both lead to higher relatedness in a way that favours the evolution of sterile helper and pure reproductive division of labour over uniform non-cooperation.

Broadly, our conceptual understanding of division of labour has been anchored to a limited number of complex systems, particularly the eusocial insects, cooperative breeders and certain obligate multicellular organisms. Our model did not incorporate a number of factors that have been argued to be important in these systems, such as haplodiploid genetics, partially overlapping generations and large group sizes[2,6,18,44–46]. Furthermore, we did not restrict our model to the extreme case of maximal group relatedness, with clonal groups formed from single cells (or family groups from lifetime monogamy). Instead, our results show that the evolution of division of labour does not require such specific life-history characteristics and can evolve in much simpler cases. More generally, there is a rich precedent in evolutionary theory of using the predictions of simple models to better understand the behaviour of complex systems[2,22,23].

Conclusion

To conclude, we found that when division of labour is favoured, it tends to adopt extreme forms, involving pure reproductives that are dependent on the helping behaviour of others. We found that helper sterility may evolve even with appreciable within-group conflict. This illustrates that division of labour is not merely a group-level adaptation that evolves to maximize group efficiency[20]. Division of labour can be favoured by kin selection at the level of the individual and play a significant role in members of social groups becoming dependent on each other. Consequently, division of labour is a driver, not a consequence, of major evolutionary transitions to higher levels of individuality, such as multicellularity and eusociality[1].

Methods

The fitness equation. We write the fitness of an individual as its expected fitness averaged across the possible phenotypes. Specifically, the neighbour-modulated (direct) fitness of a focal mutant with strategy (p, q_1, q_2) is given by:

$$\begin{aligned} W \;=\; & p(1-q_1)\left[(1-e)+e\left((1-\lambda)q_1^{\alpha}\right.\right. \\ & \left.\left.+\lambda\left(PQ_1^{\alpha}+(1-P)Q_2^{\alpha}\right)\right)\right] \\ & +(1-p)(1-q_2)\left[(1-e)+e\left((1-\lambda)q_2^{\alpha}\right.\right. \\ & \left.\left.+\lambda\left(PQ_1^{\alpha}+(1-P)Q_2^{\alpha}\right)\right)\right] \end{aligned}$$

where P, Q_1 and Q_2 are the average, others-only trait-values of social group neighbours[22,47,48]. The two terms (top two rows and bottom two rows) represent the realized fitness when of phenotype 1 (with probability p) and phenotype 2 (with probability $1-p$), respectively. Alternatively, the fitness equation may be conceptualized as the fitness of a founding individual of a social group, expressed as an expectation over the fitness of its descendants in the last generation of the social group before dispersal (haystack model). The essentiality of the trait, e, is defined as the fraction of the realized fitness benefit that arises from cooperation rather than the asocial environment. The fitness benefit from cooperation in turn is composed of the benefit from personal investment in cooperation ($(1-\lambda)(\ldots)$) and the benefit that arises from the investment of social group neighbours ($\lambda(\ldots)$). The benefit due to cooperation of social group neighbours is equal to $\sum_{i=1}^{N-1}\left(p_i q_{1,i}^{\alpha}+(1-p_i)q_{2,i}^{\alpha}\right)/(N-1)$, where i is an index of social group members that does not include the focal individual and N is the size of the social group. We approximate this as $(PQ_1^{\alpha}+(1-P)Q_2^{\alpha})$, which holds under rare mutation and weak selection (arithmetic mean is approximately equal to the geometric mean in this case).

Equilibrium analysis. We seek the evolutionarily stable strategy (ESS), (p^*, q_1^*, q_2^*), which is the strategy that, when employed by all individuals in the population, is uninvadable by a rare mutant lineage with an alternative strategy[23]. In Supplementary Section 1, we use numerical methods to determine the equilibria of the model, except in a number of special cases where we are able to solve for the equilibria analytically. An equilibrium point is defined as a joint strategy (p, q_1, q_2) for which directional selection in each trait is zero. We employ the directional selection forms developed by Taylor and Frank[47] and Brown and Taylor[48]. For example, directional selection in p is given as $W_p(p, q_1, q_2)=\frac{\partial W}{\partial p}+R\frac{\partial W}{\partial P}$, where the partial derivatives are evaluated for a monomorphic population ($p=P$, $q_1=Q_1$, $q_2=Q_2$) and R is the relatedness of interacting individuals (others only). We employ an open-model approach and assume that R is a fixed, independent parameter of the model. An equilibrium strategy is then an ESS if it is uninvadable such that rare mutants are always less fit than an arbitrary individual in the equilibrium population. In Supplementary Section 2, we use a haystack model to show that the equilibria of our model are uninvadable, and hence ESSs, with an analytical uninvadability analysis, numerical verification and individual-based simulations. In Supplementary Section 3, we use the methodology of Brown and Taylor[48] to show that all of the ESSs analysed are convergent stable, such that the population is expected to evolve towards the equilibrium in trait space.

Model assumptions. The construction of our model and its analysis relies on a set of life history, demographic and evolutionary assumptions, each of which may limit the applicability of the model in specific cases, for specific species. For example, we assumed that the population is infinite, structured into groups of fixed size, that reproduction is asexual with non-overlapping generations and that mutations are rare and lead to weak differences in selection. We also assumed that all competition is global. Taken as a whole, this constructs a model for division of labour that is only exact for very simple forms of life and we do not claim that our model makes exact predictions for division of labour in all species. However, we contend that our predictions should also hold broadly in nature when averaged across the tree of life. This will be true so long as our assumptions have not removed or rendered rigid a factor that is consistently important for the evolution of division of labour.

In some cases, factors that we have not modelled may be subsumed into the analysis. For example, although our model does not explicitly model the role of group size (N) in the evolution of division of labour, such predictions may be generated if we assume a relationship between group size and the other factors in our model. For example, in the volvocine algae, it has been argued that the efficiency benefit of specialization (α) is an increasing function of group size such that $\alpha=\alpha(N)$ and $\alpha'(N)>0$. In this case, assuming that cooperation is favoured, we recover the previously found result that increasing group size N favours division of labour[6]. Alternatively, if the benefits of cooperation are shared less equally in larger groups (lower λ), then larger groups would disfavour division of labour.

In Supplementary Tables 1 and 2, we summarize how our model compares and links to previous theoretical work on the evolution of division of labour.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Custom code used to demonstrate the uninvadability of the equilibria and the feedback effect driving extreme specialization is available at https://osf.io/w6tzk.

Data availability. The data that were generated in our equilibrium analysis are avaliable at https://osf.io/w6tzk.

Received: 5 October 2017; Accepted: 25 April 2018;
Published online: 28 May 2018

References

1. Maynard Smith, J. & Szathmary, E. *The Major Transitions in Evolution* (Oxford Univ. Press, Oxford, 1997).
2. Bourke, A. F. *Principles of Social Evolution* (Oxford Univ. Press, Oxford, 2011).
3. Boomsma, J. J. Kin selection versus sexual selection: why the ends do not meet. *Curr. Biol.* **17**, R673–R683 (2007).
4. West, S. A., Fisher, R. M., Gardner, A. & Kiers, E. T. Major evolutionary transitions in individuality. *Proc. Natl Acad. Sci. USA* **112**, 10112–10119 (2015).
5. Queller, D. C. Relatedness and the fraternal major transitions. *Phil. Trans. R. Soc. Lond. B* **355**, 1647–1655 (2000).
6. Michod, R. E., Viossat, Y., Solari, C. A., Hurand, M. & Nedelcu, A. M. Life-history evolution and the origin of multicellularity. *J. Theor. Biol.* **239**, 257–272 (2006).
7. Oster, G. F. & Wilson, E. O. *Caste and Ecology in the Social Insects*. (Princeton Univ. Press: Princeton, 1978).
8. Wilson, E. O. The ergonomics of caste in the social insects. *Am. Nat.* **102**, 41–66 (1968).
9. Willensdorfer, M. On the evolution of differentiated multicellularity. *Evolution* **63**, 306–323 (2009).
10. Rossetti, V., Schirrmeister, B. E., Bernasconi, M. V. & Bagheri, H. C. The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J. Theor. Biol.* **262**, 23–34 (2010).
11. Ispolatov, I., Ackermann, M. & Doebeli, M. Division of labour and the evolution of multicellularity. *Proc. R. Soc. B* **279**, 1768–1776 (2012).
12. Solari, C. A., Kessler, J. O. & Goldstein, R. E. A general allometric and life-history model for cellular differentiation in the transition to multicellularity. *Am. Nat.* **181**, 369–380 (2013).
13. Rueffler, C., Hermisson, J. & Wagner, G. P. Evolution of functional specialization and division of labor. *Proc. Natl Acad. Sci. USA* **109**, E326–E335 (2012).
14. Tannenbaum, E. When does division of labor lead to increased system output? *J. Theor. Biol.* **247**, 413–425 (2007).
15. Michod, R. E. Evolution of individuality during the transition from unicellular to multicellular life. *Proc. Natl Acad. Sci. USA* **104**, 8613–8618 (2007).
16. West, S. A. & Cooper, G. A. Division of labour in microorganisms: an evolutionary perspective. *Nat. Rev. Microbiol.* **14**, 716–723 (2016).
17. Arnold, K. E., Owens, I. P. & Goldizen, A. W. Division of labour within cooperatively breeding groups. *Behaviour* **142**, 1577–1590 (2005).
18. Hamilton, W. D. The genetical evolution of social behaviour. I and II. *J. Theor. Biol.* **7**, 1–52 (1964).
19. Ackermann, M. et al. Self-destructive cooperation mediated by phenotypic noise. *Nature* **454**, 987–990 (2008).
20. Gardner, A. & Grafen, A. Capturing the superorganism: a formal theory of group adaptation. *J. Evol. Biol.* **22**, 659–671 (2009).
21. Michod, R. E. Evolution of the individual. *Am. Nat.* **150**, S5–S21 (1997).
22. Frank, S. A. *Foundations of Social Evolution*. (Princeton Univ. Press: Princeton, 1998).
23. Parker, G. A. & Smith, J. M. Optimality theory in evolutionary biology. *Nature* **348**, 27–33 (1990).
24. Fisher, R. M., Cornwallis, C. K. & West, S. A. Group formation, relatedness, and the evolution of multicellularity. *Curr. Biol.* **23**, 1120–1125 (2013).
25. Flores, E. & Herrero, A. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat. Rev. Microbiol.* **8**, 39–50 (2010).
26. Herron, M. D., Hackett, J. D., Aylward, F. O. & Michod, R. E. Triassic origin and early radiation of multicellular volvocine algae. *Proc. Natl Acad. Sci. USA* **106**, 3254–3258 (2009).
27. Strassmann, J. E., Zhu, Y. & Queller, D. C. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408**, 965–967 (2000).
28. Velicer, G. J., Kroos, L. & Lenski, R. E. Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature* **404**, 598–601 (2000).
29. Veening, J.-W. et al. Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Mol. Syst. Biol.* **4**, 184 (2008).
30. Herron, M. D. & Michod, R. E. Evolution of complexity in the volvocine algae: transitions in individuality through Darwin's eye. *Evolution* **62**, 436–451 (2008).
31. Koenig, W. D. & Dickinson, J. L. *Ecology and Evolution of Cooperative Breeding in Birds* (Cambridge Univ. Press, Cambridge, 2004).
32. Hughes, W. O., Oldroyd, B. P., Beekman, M. & Ratnieks, F. L. Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* **320**, 1213–1216 (2008).
33. Cornwallis, C. K., West, S. A., Davis, K. E. & Griffin, A. S. Promiscuity and the evolutionary transition to complex societies. *Nature* **466**, 969–972 (2010).
34. Lukas, D. & Clutton-Brock, T. Cooperative breeding and monogamy in mammalian societies. *Proc. R. Soc. B* **279**, 2151–2156 (2012).
35. Bastiaans, E., Debets, A. J. & Aanen, D. K. Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. *Nat. Commun.* **7**, 11435 (2016).
36. Kuzdzal-Fick, J. J., Fox, S. A., Strassmann, J. E. & Queller, D. C. High relatedness is necessary and sufficient to maintain multicellularity in *Dictyostelium*. *Science* **334**, 1548–1551 (2011).
37. Giron, D., Dunn, D. W., Hardy, I. C. & Strand, M. R. Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. *Nature* **430**, 676–679 (2004).
38. Nonacs, P. Monogamy and high relatedness do not preferentially favor the evolution of cooperation. *BMC Evol. Biol.* **11**, 58 (2011).
39. Olejarz, J. W., Allen, B., Veller, C. & Nowak, M. A. The evolution of non-reproductive workers in insect colonies with haplodiploid genetics. *eLife* **4**, e08918 (2015).
40. Leggett, H. C., El Mouden, C., Wild, G. & West, S. Promiscuity and the evolution of cooperative breeding. *Proc. R. Soc. B* **279**, 1405–1411 (2012).
41. Davies, N. G. & Gardner, A. Monogamy promotes altruistic sterility in insect societies. *R. Soc. Open Sci.* **5**, 172190 (2018).
42. Gavrilets, S. Rapid transition towards the division of labor via evolution of developmental plasticity. *PLoS Comput. Biol.* **6**, e1000805 (2010).
43. Lehmann, L. & Rousset, F. How life history and demography promote or inhibit the evolution of helping behaviours. *Phil. Trans. R. Soc. B* **365**, 2599–2617 (2010).
44. Seger, J. Partial bivoltinism may cause alternating sex-ratio biases that favour eusociality. *Nature* **301**, 59–62 (1983).
45. Quiñones, A. E. & Pen, I. A unified model of hymenopteran preadaptations that trigger the evolutionary transition to eusociality. *Nat. Commun.* **8**, 15920 (2017).
46. Bonner, J. T. Perspective: the size-complexity rule. *Evolution* **58**, 1883–1890 (2004).
47. Taylor, P. D. & Frank, S. A. How to make a kin selection model. *J. Theor. Biol.* **180**, 27–37 (1996).
48. Brown, S. P. & Taylor, P. D. Joint evolution of multiple social traits: a kin selection analysis. *Proc. R. Soc. B* **277**, 415–422 (2010).
49. Diard, M. et al. Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature* **494**, 353–356 (2013).

Acknowledgements

The authors thank the following people for helpful discussion and comments on the manuscript: S. Levin, M. dos Santos, J. Biernaskie, A. Griffin, C. Cornwallis, P. Taylor, K. Boomsma, D. Unterwegger, K. Foster, G. Wild, A. Grafen, G. Taylor, T. Kiers and R. Fisher. We acknowledge the use of the University of Oxford Advanced Research Computing (ARC) facility in carrying out this work. G.A.C. is funded by the Engineering and Physical Sciences Research Council (EP/F500394/1).

Author contributions

G.A.C. carried out the modelling work. G.A.C. and S.A.W. conceived the study and wrote the paper. Both authors gave final approval for publication.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41559-018-0564-9.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to G.A.C.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature research

Corresponding author(s): Guy Alexander Cooper

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined. N/A

2. Data exclusions

   Describe any data exclusions. N/A

3. Replication

   Describe whether the experimental findings were reliably reproduced. N/A

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups. N/A

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis. N/A

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☒	☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☒	☐ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ A statement indicating how many times each experiment was replicated
☒	☐ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☒	☐ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☒	☐ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	All numerical analysis used Matlab R2015a. Custom code is available at osf.io/w6tzk

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	N/A

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	N/A

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	N/A
b. Describe the method of cell line authentication used.	N/A
c. Report whether the cell lines were tested for mycoplasma contamination.	N/A
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	N/A

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	N/A

Supplementary materials 4. Modelling relatedness and demography in social evolution (published paper)

doi:10.1002/evl3.69

Modeling relatedness and demography in social evolution

Guy A. Cooper,[1,2,*] Samuel R. Levin,[1,3,*] Geoff Wild,[4] and Stuart A. West[1]

[1]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

[2]E-mail: guy.cooper@zoo.ox.ac.uk

[3]E-mail: samuel.levin@zoo.ox.ac.uk

[4]Department of Applied Mathematics, University of Western Ontario, London, Ontario N6A 3K7, Canada

Received February 19, 2018

Accepted June 18, 2018

With any theoretical model, the modeler must decide what kinds of detail to include and which simplifying assumptions to make. It could be assumed that models that include more detail are better, or more correct. However, no model is a perfect description of reality and the relative advantage of different levels of detail depends on the model's empirical purpose. We consider the specific case of how relatedness is modeled in the field of social evolution. Different types of model either leave relatedness as an independent parameter (open models), or include detail for how demography and life cycle determine relatedness (closed models). We exploit the social evolution literature, especially work on the evolution of cooperation, to analyze how useful these different approaches have been in explaining the natural world. We find that each approach has been successful in different areas of research, and that more demographic detail is not always the most empirically useful strategy.

KEY WORDS: **Closed models, demography, evolutionary theory, life cycle, modeling, open models, population structure, relatedness.**

Theoretical models are often used to help explain how organisms behave in the natural world (Westneat and Fox 2010; Davies et al. 2012). In the field of social evolution, we use theoretical models to make predictions about and to ultimately understand behaviors that affect the fitness of individuals other than the actor (Hamilton 1964; Frank 1998; Bourke 2011). For example, we use models to predict when it is advantageous for individuals to cooperate; we use models to uncover the factors that contribute to the origin of selfish, altruistic, and even spiteful behaviors; and we use models to account for variation in the tendency to help both within and between species.

Perhaps the most influential model in social evolution was proposed by Hamilton (1964) and showed that genetic relatedness can be a key factor in explaining the adaptive value of social behaviors. Genetic relatedness is the probability that a social partner shares the same gene at a given locus relative to that of a random individual sampled from the population (Hamilton 1964, 1970; Grafen 1985). In large outbreeding populations, full siblings are related by ½, half-sibs by ¼, and so on (Grafen 1985). Individuals are favored to help relatives as this provides an indirect opportunity to further spread identical copies of their genes into the next generation. Over the last 50 years, relatedness has proven to be a fundamental concept for explaining social behavior across the tree of life, and theoretical models employing genetic relatedness have formed a cornerstone of social evolution (Frank 1998; Rousset 2004; West 2009; Bourke 2011).

The way in which relatedness is captured in theoretical models can be divided into two approaches, termed "open" and "closed" models (Box 1) (Taylor and Frank 1996; Frank 1998; Rousset 2004; Gardner and West 2006; Lion et al. 2011). In an open model, relatedness is left as an independent parameter that can be directly tuned by the theoretician without affecting the other features of the model. In a closed model, the modeler goes an extra step, to make specific assumptions about how population structure and life cycle determine relatedness. For example, the modeler might specify how model parameters, such as dispersal from the natal patch, the extent to which generations overlap, or

*Joint first authors.

Evolution Letters

the degree of monogamous mating impact relatedness from one generation to the next.

A potential problem with open models is that relatedness is not necessarily an independent variable (Taylor 1992a, 1992b). The factors that determine relatedness can influence other important factors. For example, patterns of dispersal and whether generations overlap can affect both relatedness and the relative marginal costs and benefits of social traits. Consequently, assuming that relatedness is an independent parameter in an open model could give misleading predictions. In contrast, closed models can take account of how different parameters are correlated, and so could be argued to be more correct or internally consistent. Closed modeling has become the most common approach in the field of social evolution, and has been suggested as the preferable method (Lehman and Rousset 2010; Lion et al. 2011). This raises the question of whether open models should be used.

Our aim is to critically analyse the utility of both open and closed approaches. Our starting point is two propositions, which we presume are widely agreed upon: (1) All models are wrong, in that they are not an exact representation of the natural world. (2) The usefulness of any model is determined by its ability to help explain the natural world. These two points are trivially true, but there has been little guidance in the literature for empirically minded theoreticians on when to develop one type of model over the other. We first examine the theoretical trade-offs of each approach and consider how they may be appropriate for different empirical questions. We then consider a few areas where open and closed models have been developed, including cooperation, sex allocation, and dispersal. We evaluate the success of each approach in explaining empirical patterns in these areas, to see if any lessons can be drawn for future research.

BOX 1: Open and closed: A toy model

We develop a simple model of public goods, first with an open and then a closed approach, to illustrate the two methods. We model the most general form of a public good, following Hamilton (1964), Taylor (1992a, 1992b), and Frank (2010). We take an inclusive fitness approach because the fitness derivations are simpler in this case, though an equivalent direct (neighbor modulated) fitness approach can be found in Taylor et al. (2007) and Levin and West (2017b).

Open Model: Some organism, such as a microbe, produces some costly public good, the benefits of which are shared between its social partners and itself. Examples in nature of public goods include the production and release of molecules by bacteria that scavenge for iron or digest protein (Griffin et al 2004; Diggle et al 2007). Because the production of the public good is costly to the individual, we might expect natural selection to favor individuals that do not incur the cost of production, but reap the benefits of good-producing social partners. Thus, we are interested in the conditions that would favor the evolution of the public good producing trait.

We assume an infinite population of individuals subdivided into social groups of size N (the infinite island model). Individuals can produce the public good at some private fecundity cost, c, which provides some fecundity benefit, b, to all individuals on the patch (including the focal individual). Hamilton (1964, 1970) showed that a trait will spread if its inclusive fitness effect, W_{IF}, is greater than *0* ($W_{IF} > 0$), where the inclusive fitness effect of an actor's trait is its effect on all individuals in the population, weighted by relatedness of the actor to those affected individuals (including the actor itself), or "recipients." In this case, the trait has a negative cost to the actor (with relatedness 1), and the relatedness to recipients is *r*, the average whole group relatedness in a social group (as opposed to others-only relatedness). Thus, the trait will spread if:

$$rb - c > 0,$$

which is a simple form of Hamilton's (1964) rule with b and c as simple additive fitness effects, as opposed to the general, regression form of Hamilton's rule (Gardner et al. 2011b). This is an open model, in which the mechanism by which *r* is generated is undefined. Positive relatedness in this model could come about through limited dispersal, kin recognition, partner choice, or any other process that generates genetic correlations within social groups. However, if *r* is correlated with the other model parameters (*b* and *c*), the predictions of this model might not be very useful for explaining variation in nature.

Closed Model: We might, for example, be interested in the case in which relatedness is generated through limited dispersal. We can capture this by incorporating a new parameter, *d*, which measures the proportion of offspring that disperse from their natal social group (with a fraction *(1-d)* remaining in the group). Following Taylor (1992a), we must now take into account not only the offspring produced as a direct result of public goods production, but also those offspring indirectly displaced as a result of the cooperative trait. An individual that expresses the public good trait incurs a fecundity cost, *c*, with relatedness 1, and provides a fecundity benefit, *b*, to recipients whose average relatedness is *r*. These extra *(b – c)* offspring remain in the social group with probability *(1 – d)*, in which case the individuals they displace are also native with probability *(1 – d)*, and therefore have relatedness r. The overall inclusive fitness effect, then, is

$$W_{IF} = rb - c - r(1-d)^2(b-c).$$

The above is still an open model, assuming independence between relatedness and model parameters. This illustrates that in principle, up until this point open and closed models can incorporate the same amount of demographic detail (though in practice, open models often do not). Taylor (1988, 1992a) showed how we can close the model by making additional assumptions. Specifically, he calculated relatedness in terms of the demographic parameters of the model (d & N). We can do this by writing the following population genetic recursion for the change in relatedness in a social group from one generation to the next:

$$r_{t+1} = 1/N + r_t(1-d)^2(N-1)/N.$$

Where the first term is the chance that two randomly sampled individuals on the patch are the same individual, and have relatedness one, and the second term is the chance they are different individuals both native to the patch, and therefore have the relatedness from the previous generation. Solving for the equilibrium value of relatedness, and plugging into the inclusive fitness effect above, we find the condition for the trait to spread is:

$$b/N > c.$$

This is Taylor's classic result—that the dispersal rate has no impact on whether the trait will spread.

Extensions: we can extend this closed model a number of ways to look at the impact of different life histories and explicit demographic parameters (Table 2). We do this by rewriting the fitness function and recalculating our estimate of relatedness accordingly. As one example, Taylor and Irwin (2000) allowed for overlapping generations by including a parameter s, the probability that a parent survives into the next generation. The inclusive fitness effect becomes:

$$W_{IF} = (1-s)[(rb-c) - r(1-d)^2(b-c).$$

Plugging in the equilibrium relatedness value, calculated in terms of s, d, and N, the condition for the public good trait to evolve becomes:

$$b/c > N - (N-1)[(2s(1-d))/((2-d)(1+s))].$$

The Scale of Competition

Open models can be used to provide an alternate way to look at the factors that arise in closed models (Frank 1998, Gardner and West 2006). For example, Frank (1998) developed a model for incorporating competition into an open model, by subsuming the scale of competition into benefit term of Hamilton's rule:

$$RB - C > 0$$

Where $R = r$, C = c, and B = b – a(b – c), and a is the proportion of competition that happens locally.

Queller (1994) developed a similar approach in which competition is subsumed into the relatedness parameter:

$$RB - C > 0$$

Where B = b, C = c, and $R = (r - ar)/(1 - ar)$, and therefore relatedness is not to an average member of the population but to an average competitor. Both the Queller (1994) and Frank (1998) approaches recover Taylor's (1992a) result as a specific case (see Gardner and West (2006) for further discussion).

The Trade-offs of Open and Closed Models

Open and closed modeling approaches differ in how they treat relatedness. Across nature, there is a wide diversity of life cycles and demographic structures that can generate relatedness between interacting individuals (Hamilton 1964; Frank 1998; Rousset 2004). Some well-characterized examples include:

1. Kin discrimination–if individuals can somehow distinguish relatives from nonrelatives and preferentially direct cooperation toward them, then this can generate positive relatedness between actor and recipient (Sharp et al. 2005; Mehdiabadi et al. 2006).
2. Dispersal patterns–limited dispersal, or dispersing as groups of relatives, can keep relatives together and hence generate positive relatedness between interacting individuals, in the absence of any kin discrimination (Hamilton 1964).
3. Mating patterns–monogamy or lower levels of polyandry can increase the relatedness between interacting siblings (Boomsma 2007; Hughes et al. 2008; Cornwallis et al. 2010, 2017; Lukas and Clutton-Brock 2012a).

OPEN MODELS

An open model is agnostic about which of the above factors (or others) are responsible for the generation of relatedness between individuals. Instead, relatedness is deliberately left as an independent factor that can be tuned directly by the modeler. The benefit of this approach is that it can generate predictions that should hold across many systems, regardless of which specific demographic processes are responsible for relatedness between interacting individuals. Thus, if the model predicts that investment in a public good will increase for higher relatedness, then this should hold just as well in systems that employ kin discrimination, limited dispersal or monogamous mating in the generation of relatedness.

The downside of an open approach is that relatedness is not necessarily independent of other factors. For example, relatedness can be an important driver of the evolution of dispersal, but relatedness also crucially depends upon dispersal (Taylor 1988; Frank 1998). Open models miss such feedbacks (West et al. 2002; Lehmann and Rousset 2010). Consequently, open models may gain widespread applicability, but at a cost of demographic precision.

CLOSED MODELS

Closed models

In contrast, a closed model specifies the precise way in which population dynamical processes generate genetic relatedness (Table 2). In doing so, concrete assumptions must be made about the exact life cycle and demography of the system and how these factors contribute to the relatedness of interacting individuals.

The benefit of a closed-model approach is that it allows a specific question to be answered about a characterized system, in which the processes that generate relatedness are known. Any feedback effects between parameters or traits of the model with the underlying genotypic assortment in the population are captured by the model. Furthermore, because the population-genetic assumptions about relatedness are clearer, closed models lend themselves to tweaking and altering assumptions or parameters in a way that allows theoreticians to build a family of related models, for which the intermodel relationships are apparent (Table 2).

However, the final step of closing a model involves determining precisely how a specific demography generates relatedness. Consequently, any conclusions drawn might only be applicable to that or a limited number of scenarios. This gives a precise solution, but it might be precisely irrelevant to what occurs in the real world. In fact, the way that relatedness arises in natural systems is frequently not well understood, arising from a convoluted combination of factors and processes. As such, the additional demographic assumptions that make closed models solvable are sometimes so idealized that they may add less realism to the model than might otherwise be expected (Taylor 1992a, 1992b; Gardner and West 2006; Lehman and Rousset 2010; Table 2). Consequently, closed models gain precise demographic detail, but at a cost of broader applicability.

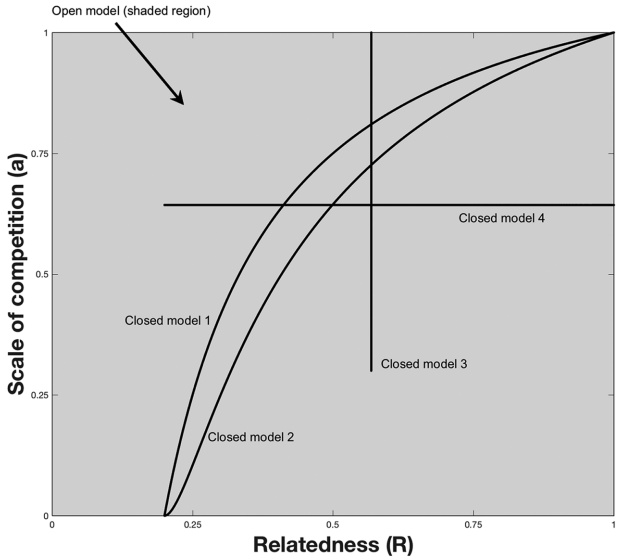


Figure 1. The relation between open and closed models. Frank (1998) developed an open model to show how local competition could reduce selection for cooperation between relatives. He used a parameter "*a*" to measure the scale at which density-dependent competition occurs, which can range from completely global (a = 0) to completely local (a = 1). In this figure, *a* is plotted against relatedness (*R*). Frank allowed these two variables to vary independently, and so his model encompasses the entire plane (shaded gray). In a closed model, we assume a specific demography and life history, and this causes *a* and *R* to be correlated in a specific way, leading to a particular curve in the plane (dark lines). For example, Closed model 1 is Taylor's 1992a model, closed model 2 is Taylor and Irwin's (2000) overlapping generations model, and closed models 3 and 4 are Gardner and West's 2006 budding dispersal model, for a fixed budding dispersal rate and range of migration rates, and a fixed migration rate and range of budding dispersal rates, respectively. Adapted from Gardner and West (2006).

OPEN VERSUS CLOSED

The differences between open and closed models can be illustrated graphically. Figure 1 graphs the relatedness (R) between interacting individuals versus the extent to which density dependent competition is at the scale of the local patch (*a;* Frank 1998). An open model can allow both these parameters to vary independently (the entire parameter space). A closed model determines how these parameters are related for a specified demography (one line on the figure). There are many different possible demographic scenarios and corresponding closed models (different lines on the figure). We provide some examples, which illustrate how different demographic assumptions can qualitatively change whether and how R and a are linked. This figure also illustrates how an open model can be used as a "meta-model" to examine how different closed models work and relate to each other (Frank 1998).

While there is a rough correlation between "open and closed" and "simple and complex," this is not always the case. In principle, closed models are nested within open models–up until the point of specifying relatedness, a closed model is open (Box 1). However, in practice, not all open models are one step away from being a closed model as the demography that determines relatedness and is required to close the model may not be specified at all (Wild 2011). Open models may instead include other ecological factors or otherwise unlinked demographic details and thus can be arbitrarily complex. Furthermore, in closed models, the interplay between different factors can sometimes lead to simpler predictions, as some parameters drop out of the analysis (Pen and Weissing 2000). Consequently, the difference between open and closed models may often be less of a distinction in complexity rather than a differing emphasis in the kinds of details that are included.

The above is a conceptual discussion of the relative trade-offs of open and closed modeling. However, the utility of different theoretical approaches is not a philosophical question, it is something that needs to be empirically tested. What matters is the interplay

between theory and data. Luckily, such an analysis is possible, via the extensive theoretical and empirical literature on the evolution of cooperation.

The Evolution of Cooperation: An Illustrative Example

A behavior or trait is defined as cooperation if it provides a benefit to another individual, and has evolved at least partially because of this benefit (West et al. 2007b). Cooperation poses an evolutionary problem because, all else being equal, it would reduce the relative fitness of the co-operator, and hence be selected against. There is a rich theoretical and empirical literature explaining the factors that can favor cooperation (Sachs et al. 2004; West et al. 2007a; Bourke 2011).

OPEN MODELS OF COOPERATION

A potential explanation for cooperation is that it is directed toward relatives, who also carry the gene for cooperation. By helping a relative reproduce, an individual is still passing copies of its genes to the next generation, just indirectly. This process, which is usually termed kin selection, was first modeled by Hamilton (1964) (Box 1). Hamilton showed that an altruistic cooperative trait will evolve if the fitness cost to the cooperator (C) is smaller than the fitness benefit (B) to the recipient, where the benefit to the recipient is weighted by the relatedness (R) of the cooperator to the recipient: $RB - C > 0$.

This result, known as Hamilton's rule, is an open model. Relatedness is a parameter (R) that is treated as independent of the other parameters of the model. There is no specification of how a positive R arises. As such, there are a number of population—and individual-level mechanisms that could generate a given R value.

Hamilton's rule has been employed to explain a wide range of traits across the tree of life (Table 1). It has been used to explain behavior, and variation in behavior, across diverse taxa, including bacteria, slime moulds, insects, birds, and mammals. The behaviors considered include many different forms of cooperation, policing, division of labor, dispersal, and harming behaviors such as killing or cannibalism. Furthermore, this includes cases where positive relatedness, or variation in relatedness, arises from a variety of factors, including limited dispersal, level of polyandry (promiscuity), kin discrimination and how groups are formed. In many cases, open models for more specific traits have also been developed (Table 1).

Closed models of cooperation

The open models discussed above black-boxed the mechanism that generated relatedness, and implicitly assumed that relatedness was independent of other model parameters. Over the last 30 years, many modelers interested in cooperation have instead employed closed models (Table 2).

Hamilton (1964) recognized that population viscosity via limited dispersal is a key mechanism for generating the positive relatedness values that can favor cooperation in Hamilton's rule. At the same time, however, limited dispersal can also increase competition between relatives, which reduces the relative benefit of helping relatives (Hamilton 1971, 1975). It is possible to put this local competition into an open model by adding an extra independent parameter or parameters (Grafen 1984; Frank 1998; Grafen and Archetti 2008). For example, RB-C-R_2D_2, where R_2 is the average relatedness between the actor and the individuals that suffer from increased competition and D_2 is the cost to these individuals (Grafen 1984). However, when parameters such as R and R_2 or B and D_2 are determined by the same factors, they will be correlated. Consequently, keeping them as independent parameters could give misleading predictions. For example, if limited dispersal increases both R and R_2, then we might not expect a higher relatedness (R) to lead to higher cooperation.

Taylor (1992a) developed a closed model of cooperation that considered the explicit effects of social group size and dispersal rates. He then estimated the value of relatedness as generated by the specific life-history details of the model. In a landmark result, he found that the dispersal rate had no influence on the evolution of cooperation. In Taylor's model, the effect of increased relatedness and competition exactly cancel. As such, Taylor's closed model predicted that a decrease in dispersal (and therefore an increase in relatedness) would not favor cooperation as predicted by the simple form of Hamilton's rule. As well as this specific result, for that exact life history, Taylor's model makes a general point about how we need to consider both cooperation and competition between relatives.

Taylor's model has since been expanded into a number of other closed models that tweak the life history in some manner (Table 2). In many of these cases, the specific life cycle allows limited dispersal to increase relatedness (R), without being exactly cancelled by a decreased benefit to relatives (B). Consequently, in these models, limited dispersal can favor cooperation. For example, Taylor and Irwin (2000) found that overlapping generations increase relatedness without inflating the costs of competition. This happens because there is a population-level mechanism (parent survival) for genetic associations to accrue in the absence of extra offspring remaining on the patch and competing (Box 1).

However, these closed models have had relatively little impact on our empirical understanding of specific biological cases. There is only one empirical example from the natural world where the data suggests that the influence of dispersal rates on relatedness and competition exactly cancel out–competition for mates between male fig wasps (West et al. 2001). The closed models stimulated experimental evolution studies in bacteria,

Table 1. **Examples of some of the phenomena where an open model approach (Hamilton's rule) has helped us understand biological phenomena.**

Taxa	Trait/Phenomena explained	Cause of variation in R	Empirical approach	More specific open models
Bacteria	Public goods (extracellular factors)	Dispersal pattern	Experimental evolution (Griffin et al. 2004)	Brown 1999; West and Buckling 2003; Dionisio and Gordo 2006; Frank 2010
Bacteria	Quorum sensing	Dispersal pattern	Experimental evolution (Diggle et al. 2007; Rumbaugh et al. 2012; Pollitt et al. 2014; Popat et al. 2015)	Brown and Jonstone 2001
Bacteria	Killing (bacteriocins)	Kin discrimination, dispersal pattern	Experimental (Inglis et al. 2009)	Gardner et al. 2004
Bacteria	Symbiotic benefit	Dispersal pattern (transmission)	Comparative (Fisher et al. 2017)	Frank 1996a
Birds and mammals	Cooperative breeding	Level of polyandry	Comparative (Cornwallis et al. 2010; 2017; Lukas and Clutton-Brock 2012a, 2012b)	Charnov 1981
Birds and mammals	Cooperation	Kin discrimination	Observational, experimental, comparative (Komdeur 1994; Russell and Hatchwell 2001; Griffin and West 2003; Komdeur et al. 2004; Sharp et al. 2005; Cornwallis et al 2009)	–
Fungus	Cooperation	Group formation, kin discrimination	Experimental evolution (Bastians et al. 2016)	–
Insects	Eusociality	Level of polyandry	Comparative (Hughes et al. 2008)	Charnov 1978, 1981; Gardner et al. 2011a; Alpedrinha et al. 2013, 2014; Rautiala et al. 2014; Liao et al. 2015,
Insects	Policing	Level of polyandry	Experimental, Comparative (Wenseleers and Ratnieks 2006a, 2006b; Ratnieks et al. 2006)	Ratnieks 1988; Wenseleers et al. 2004a, 2004b
Insects	Killing	Haplodiploidy, dispersal pattern, kin discrimination	Observational, experimental (Grbic et al. 1992; Giron et al. 2004a, 2004b)	–
Insects	Reproductive restraint	Level of polyandry	Observational, comparative (Wensellers and Ratnieks 2004)	Wenseleers et al. 2003, 2004a
Salamanders	Cannibalism	Kin discrimination	Experimental (Pfennig and Collins 1993; Pfennig et al. 1994, 1999)	–

(*Continued*)

Table 1. Continued.

Taxa	Trait/Phenomena explained	Cause of variation in *R*	Empirical approach	More specific open models
Slime moulds	Fruiting bodies	Dispersal pattern, kin discrimination	Observational, experimental evolution, genomic (Mehdiabadi et al. 2006; Gilbert et al. 2007; Kuzdzal-Fick et al. 2011; Ostrowski et al. 2015; Noh et al. 2018)	–
Social groups of cells (across taxa)	Division of labor, sterile cells	Dispersal pattern	Comparative (Fisher et al. 2013)	Cooper and West 2018

Our list is illustrative, not exhaustive, and we provide examples of the consequences of variation in only a single parameter (*R*). More specific open models are often constructed for specific traits. In many cases, some form of Hamilton's rule emerges as a prediction and is useful for interpreting these models (Taylor and Frank 1996; Frank 1998). For some other traits, such as sex allocation, the results are still interpreted with kin selection, but Hamilton's rule per se is less useful for interpretation. Studies focusing on the consequences of variation in other parameters (B, C), and whether Hamilton's rule is satisfied, are reviewed elsewhere (Bourke 2011, 2014).

examining how patterns of dispersal can influence both relatedness and competition (Griffin et al. 2004, Kümmerli et al. 2009). However, these studies can be seen as "wet simulations" that validate theory, but do not actually measure the consequences of competition in nature. Further, the role of demographic details has been discussed but rarely tested in a number of taxa, including RNA replicators, birds, and killer whales (Hatchwell 2009; Johnstone and Cant 2010; Croft et al. 2017; Levin and West 2017a).

OPEN VERSUS CLOSED

Why have open models been more useful for explaining specific empirical examples of cooperation? We suggest seven, nonmutually exclusive possibilities: (i) a closed model specifies a certain demography, narrowing the organisms to which it can be applied; (ii) closed models include an additional layer of demographic detail, which can make them more complex, and harder for empiricists to apply (or at least, they appear to); (iii) open models can offer intuitive heuristics, like Hamilton's rule, which can be applied broadly, generate simple predictions, and facilitate interpretation of results; (iv) open models make predictions in terms of *R*, which will often be a relatively easy parameter to measure; (v) open models disentangle causal effects in similar way to experiments that try to manipulate single factors while keeping everything else fixed; (vi) open models can focus on other biological details of potential interest, rather than demography (e.g., partner sanctions, or how cooperative benefits are shared; West et. all 2002; Cooper and West 2018); and (vii) there may not be enough two-way interactions between those developing the theory and those collecting the data.

The utility of the different approaches can also be illustrated by imagining a hypothetical scenario in which theoretical work on cooperation had started with Taylor's (1992a) closed model. In this case, we would have been left with the prediction that limited dispersal (higher relatedness) does not favor cooperation. Empirically this is clearly not the case, as limited dispersal appears to play a key role in favoring cooperation in a broad range of taxa (Table 1). But, at the same time, Taylor's model has been incredibly influential in its own right. The point is that Taylor's closed model was useful when discussed against an open model (Hamilton's rule). Hamilton's rule said relatedness matters, and it clearly does (Table 1). Taylor's model showed that, in certain cases, things could be more complicated as competition can reduce selection or even negate selection for cooperation between relatives. This helped us explain the data from fig wasps and stimulated experiments on bacteria (West et al. 2001; Griffin et al. 2004; Kümmerli et al. 2009), and led to a large body of theoretical work (Lehmann and Rousset 2010; Van Cleve and Lehman 2013; Van Cleve 2015; Peña et al. 2015). Furthermore, the combination of open and closed models in this area also spurred work on how local competition can favor spiteful harming behaviors (Gardner and West 2004; Gardner et al. 2004; 2007; Lehmann et al. 2006).

Beyond Cooperation

How useful have open and closed models been more generally? Another area of social evolution where there has been productive interplay between theory and data is the study of how organisms allocate resources to male and female offspring, termed sex

Table 2. Examples of the ways that Taylor's (1992a) model has been extended to incorporate additional biological details (nonexhaustive).

Theoretical models	Process modeled	When does limited dispersal favours cooperation?
Taylor 1992a	Patch elasticity	Always
Taylor and Irwin 2000, Irwin and Taylor 2001, Levin and West 2017b	Overlapping generations	When generations overlap
Gardner and West 2006, Lehmann et al. 2006, Lehmann et al. 2007, Traulsen and Nowak 2006	Budding dispersal	When individuals are more likely to disperse together than singly (budding).
Rogers 1990	Selective emigration	If altruists are more likely to emigrate
Gardner 2010, Johnstone and Cant 2008	Sex-specific dispersal	When the sex with higher variance in fitness is (slightly) more likely to disperse
Lehmann et al. 2008, Johnstone 2008	Caste-specific dispersal	When different castes (e.g. queen and worker) have different dispersal rates, reproductive values, and dispersal timings
Alizon and Taylor 2008	Empty sites	When there are empty sites on patches
El Mouden and Gardner 2008	Conditional helping	When co-operators adjust their behaviour conditional on whether they disperse
Taylor 1992b, Kelly 1992, Queller 1994, Gardner and West 2006	Various timings of cooperation and competition	Under some but not all demographic timing schemes
Yeh and Gardner 2012	Different ploidies	Under some but not all ploidies
Rodrigues and Gardner 2012, 2013a, b	Heterogeneity in patch quality, group size, and individual quality	When patches vary spatially and temporally in patch quality and group size, and (under some circumstances) when individuals vary in quality
Perrin and Lehmann 2001	Kin discrimination	When individuals can actively discriminate kin

We focus here on analytical models (rather than simulations), as these allow us to the see the explicit role of different parameters. We focus on island models, as opposed to spatially explicit models (e.g., lattice or stepping stone), as the added mathematical complexity of these models makes it harder to interpret parameter relationships, without necessarily revealing patterns that can't already be identified in simpler island models (Lehmann and Rousset 2010). A number of other models have used different approaches (e.g., lattice models, cellular automata, evolution on graphs) to identify a number of other factors that can alleviate the effects of local competition (e.g., van Baalen and Rand 1998; Mitteldorf and Wilson 2000; Ohtsuki et al. 2006; Lehmann et al. 2006; Grafen 2007; Taylor et al. 2007; Lion and Gandon 2009).

allocation (West 2009). Within this area, the two relevant success stories are: (1) local mate competition (LMC)–how population structuring, with competition for mates between related males, selects for female biased sex ratios (Hamilton 1967); (2) sex allocation driven by relatedness asymmetries in haplodiploid social insects (Trivers and Hare 1976; Boomsma and Grafen 1991). Closed and open models have driven research in these two areas respectively, demonstrating that, in different fields, one approach has sometimes been more useful than the other.

Hamilton (1967) showed that if n diploid females lay eggs on a patch, if mating then occurs on this patch, and if only the females disperse to compete globally, then the evolutionarily stable strategy is to invest a fraction $(n\text{-}1)/2n$ of resources into female offspring. The beauty of this closed model is that it is an excellent approximation of the life history of many species, and leads to a prediction in terms of one parameter that is often relatively easy to measure (n). A closed model works so well here, because clear morphological features, such as nondispersing wingless males, enforce life-history features that facilitate mathematical simplifications. Hamilton's LMC model has proved extremely useful for explaining variation in sex allocation, both within and between species (West 2009). Furthermore, theory has been extended in numerous directions to account for life history and demographic details relevant to certain species (West 2009). Alternative open formulations of Hamilton's LMC equation are possible, which focus on the relatedness between male and female offspring on a patch, but these can be less easy to apply (Frank 1998; Nee et al. 2002).

Boomsma and Grafen (1991) showed that, in haplodiploid social insects, workers are favored to adjust the colony sex allocation in response to the relatedness structure within their colony. They

produced an open model, and outlined how relatedness structure could be determined by a number of demographic factors, including queen mating rate, queen number, worker reproduction and queen replacement. Their model is able to explain considerable variation in sex allocation, between colonies (split sex ratios), in response to these factors (West 2009). A single open model could be applied across, and therefore unify, a number of different scenarios, where different features of the demography drive "split sex ratios." Together, these examples from sex allocation highlight that, for distinct empirical questions, different approaches have been more useful.

There are other areas where open or closed models have been more important for the development of theory. For example, closed models have dominated theoretical work on the evolution of dispersal, because the dispersal rate is both the trait under selection and the determinant of relatedness (Taylor 1988; Frank 1998; Gandon 1999; Gandon and Michalakis 1999; Gandon and Rousset 1999; Rousset 2004). Another example is the evolution of virulence, where early models tended to be open whereas later models are predominately closed (Frank 1996b; Gandon and Michalakis 2000; Wild et al. 2009; Alizon and Lion 2011; Lion 2013). However, neither of these fields has led to a similar interplay between theory and data, possibly because most of the theory was not developed to address specific empirical patterns (Crespi and Taylor 1990; Innocent et al. 2010).

Finally, there are also parameters other than relatedness that could be left open or closed. For example, in models where populations are structured into different classes—such as age, sex, or size—reproductive values are usually treated as closed. However, open models could be developed in these cases by employing a conservation of reproductive value criterion. Because total reproductive value of the population is constant, an increase in the reproductive value of one individual necessitates exact compensatory changes in the reproductive value of others, allowing the modeler to keep this as an open parameter (e.g., Wild and West 2007). Exactly how our analysis extends to these other questions remains unclear.

Guidelines

An obvious take home is that the different approaches have different utilities. But this is a bit vague and obvious. Can a summary of our above discussion provide more specific guidelines?

Open models have proved more useful when we want to consider cases where multiple demographic and life-history details can influence relatedness. For example, how limited dispersal, kin discrimination, and female mating rate influence the evolution of cooperation, or how queen mating rate, queen number, and queen replacement influence the evolution of split sex ratios (Hamilton 1964; Boomsma and Grafen 1991). In these cases, an open model can be applied broadly across diverse taxa, with very different life cycles. In addition, open models have been useful for providing conceptual unification, and intuitive heuristics for guiding empirical work.

Closed models have proved particularly useful when a single demographic factor is more universally important. For example, how the number of females laying eggs per patch influences sex allocation (Hamilton 1967). In such cases, a closed model can be applied broadly across different taxa, which share this key aspect of their life cycle. In addition, closed models have been useful conceptually for disentangling the roles of different demographic parameters.

More generally, with all these considerations, the emphasis should always be on the interplay between theory and data, and how the theory will be used to help us explain the natural world. When developing theory, there are a number of empirically motivated questions to be asked. What aspect of the empirical data can't be explained by existing theory and needs a new model? What are the parameters that empirical work suggests need more attention? Do we want to make broad predictions across species with different life cycles, or for a single species with a specific life cycle? The advantage of more empirically minded development of theory is clearly illustrated by the success of closed models developed to examine sex allocation (local mate competition), compared to those for cooperation and dispersal. In particular, the extensions of basic local mate competition theory have proven very useful precisely because their development was driven by cases where the data and/or life-history assumptions did not fit existing theory (West 2009).

Conclusions

To conclude, open and closed models are complementary and not competing approaches. Ultimately, we must ask what the modeler is prepared to give up, and what they want to gain, which will depend on the modeler's empirical aim. Sylvain Gandon pointed out to us that an analogy here can be made with the analysis of statistical data. If the addition of an extra variable does not significantly improve the explanation of the data, then the more detailed model, with that extra variable, can be a less good model, as judged by statistical measures such as AIC. An important goal should be to develop a model with the minimal level of detail required to answer a specific biological question (May 2004). Evaluating whether to use an open or closed model is then simply a matter of determining where that minimal level of detail falls with respect to demography and population structure.

Finally, this debate touches on a recurring theme in behavioral and evolutionary ecology, where there are numerous examples of different potential approaches. Some examples include

population genetics versus game theory, general versus specific models in game theory, or experimental studies on a specific species versus across species comparative studies (Harvey and Purvis 1991; Parker and Maynard Smith 1990; Davies et al. 2012). All of these cases have generated arguments that one approach is "better" or "more correct" than the other whereas, in reality, the different methodologies have different strengths and weaknesses and are each appropriate in different scenarios.

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript equally.

ACKNOWLEDGMENTS

We thank Sylvain Gandon, Miguel dos Santos, Andy Gardner, Michael Cant, and one anonymous reviewer for their helpful comments and discussion.

LITERATURE CITED

Alizon, S., and P. Taylor. 2008. Empty sites can promote altruistic behavior. Evolution 62:1335–1344.

Alizon, S., and S. Lion. 2011. Within-host parasite cooperation and the evolution of virulence. Proc. R Soc. Lond. B Biol. Sci. 278:3738–3747.

Alpedrinha, J., A. Gardner, and S. A. West. 2014. Haplodiploidy and the evolution of eusociality: worker revolution. Am. Nat. 184:303–317.

Alpedrinha, J., S. A. West, and A. Gardner. 2013. Haplodiploidy and the evolution of eusociality: worker reproduction. Am. Nat. 182:421–438.

Bastiaans, E., A. J. Debets, and D. K. Aanen. 2016. Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. Nat. Comm. 7:11435.

Boomsma, J. J., and A. Grafen. 1991. Colony-level sex ratio selection in the eusocial Hymenoptera. Journal of Evolutionary Biology 4:383–407.

Boomsma, J. J. 2007. Kin selection versus sexual selection: why the ends do not meet. Curr. Biol. 17:R673–R683.

Bourke, A. F. 2011. Principles of social evolution. Oxford Univ. Press, Oxford.

Bourke, A. F. 2014. Hamilton's rule and the causes of social evolution. Phil. Trans. R Soc. B 369:20130362.

Brown, S. P. 1999. Cooperation and conflict in host–manipulating parasites. Proc. R. Soc. Lond. B Biol. Sci. 266:1899–1904.

Brown, S. P., and R. A. Johnstone. 2001. Cooperation in the dark: signalling and collective action in quorum-sensing bacteria. Proc. R Soc. Lond. B Biol. Sci. 268:961–965.

Charnov, E. L. 1978. Evolution of eusocial behavior: offspring choice or parental parasitism? J. Theoret. Biol. 75:451–465.

Charnov, E. L. 1981. Kin selection and helpers at the nest: effects of paternity and biparental care. Anim. Behav. 29:631–632.

Cooper, G. A., and S. A. West. 2018. Division of labour and the evolution of extreme specialisation. Nat. Ecol. Evol. 2:1161–1167.

Cornwallis, C. K., C. A. Botero, D. R. Rubenstein, P. A. Downing, S. A. West, and A. S. Griffin. 2017. Cooperation facilitates the colonization of harsh environments. Nat. Ecol. Evol. 1:0057.

Cornwallis, C. K., S. A. West, K. E. Davis, and A. S. Griffin. 2010. Promiscuity and the evolutionary transition to complex societies. Nature 466:969.

Cornwallis, C. K., S. A. West, and A. S. Griffin. 2009. Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. J. Evol. Biol. 22:2445–2457.

Crespi, B. J., and P. D. Taylor. 1990. Dispersal rates under variable patch density. Am. Nat. 135:48–62.

Croft, D. P., R. A. Johnstone, S. Ellis, S. Nattrass, D. W. Franks, L. J. Brent, et al. 2017. Reproductive conflict and the evolution of menopause in killer whales. Curr. Biol. 27:298–304.

Davies, N. B., J. R. Krebs, and S. A. West. 2012. An introduction to behavioural ecology. John Wiley & Sons, Hoboken, New Jersey.

Diggle, S. P., A. S. Griffin, G. S. Campbell, and S. A. West. 2007. Cooperation and conflict in quorum-sensing bacterial populations. Nature 450:411.

Dionisio, F., and I. Gordo. 2006. The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. Evol. Ecol. Res. 8:321–332.

El Mouden, C., and A. Gardner. 2008. Nice natives and mean migrants: the evolution of dispersal-dependent social behaviour in viscous populations. J. Evol. Biol. 21:1480–1491.

Fisher, R. M., C. K. Cornwallis, and S. A. West. 2013. Group formation, relatedness, and the evolution of multicellularity. Curr. Biol. 23:1120–1125.

Fisher, R. M., L. M. Henry, C. K. Cornwallis, E. T. Kiers, and S. A. West. 2017. The evolution of host-symbiont dependence. Nature Communications 8:15973.

Frank, S. A. 1996a. Host–symbiont conflict over the mixing of symbiotic lineages. Proc. R. Soc. Lond. B 263:339–344.

Frank, S. A. 1996b. Models of parasite virulence. Quart. Rev. Biol. 71:37–78.

Frank, S. A. 1998. Foundations of social evolution. Princeton Univ. Press, Princeton.

Frank, S. A. 2010. A general model of the public goods dilemma. J. Evol. Biol. 23:1245–1250.

Gandon, S. 1999. Kin competition, the cost of inbreeding and the evolution of dispersal. J. Theoret. Biol. 200:345–364.

Gandon, S., and Y. Michalakis. 1999. Evolutionarily stable dispersal rate in a metapopulation with extinctions and kin competition. J. Theoret. Biol. 199:275–290.

Gandon, S., and Y. Michalakis. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. Proc. R Soc. Lond. B Biol. Sci. 267:985–990.

Gandon, S., and F. Rousset 1999. Evolution of stepping-stone dispersal rates. Proc. R Soc. Lond. B Biol. Sci. 266:2507–2513.

Gardner, A., and S. A. West. 2006. Demography, altruism, and the benefits of budding. J. Evol. Biol. 19:1707–1716.

Gardner, A. 2010. Sex-biased dispersal of adults mediates the evolution of altruism among juveniles. Journal of Theoretical Biology 262:339–345.

Gardner, A., J. Alpedrinha, and S. A. West. 2011a. Haplodiploidy and the evolution of eusociality: split sex ratios. The American Naturalist 179:240–256.

Gardner, A., S. A. West, and G. Wild. 2011b. The genetical theory of kin selection. Journal of Evolutionary Biology 24:1020–1043.

Gardner, A., I. C. Hardy, P. D. Taylor, and S. A. West. 2007. Spiteful soldiers and sex ratio conflict in polyembryonic parasitoid wasps. Am. Nat. 169:519–533.

Gardner, A., S. A. West, and A. Buckling. 2004. Bacteriocins, spite and virulence. Proc. R Soc. Lond. B Biol. Sci. 271:1529–1535.

Gilbert, O. M., K. R. Foster, N. J. Mehdiabadi, J. E. Strassmann, and D. C. Queller. 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. Proc. Natl. Acad. Sci. 104:8913–8917.

Giron, D., D. W. Dunn, I. C. Hardy, and M. R. Strand. 2004a. Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. Nature 430:676.

Giron, D., S. Pincebourde, and J. Casas. 2004b. Lifetime gains of host-feeding in a synovigenic parasitic wasp. Physiological Entomology 29:436–442.

Grafen, A. 1984. Natural selection, kin selection and group selection. Behavioural Ecology: An Evolutionary Approach 2:62–84.

Grafen, A., and M. Archetti. 2008. Natural selection of altruism in inelastic viscous homogeneous populations. Journal of Theoretical Biology 252:694–710.

Grafen, A. 1985. A geometric view of relatedness. Oxford Surv. Evol. Biol. 2:28–89.

Grafen, A. 2007. An inclusive fitness analysis of altruism on a cyclical network. J. Evol. Biol. 20:2278–2283.

Grbić, M., P. J. Ode, and M. R. Strand. 1992. Sibling rivalry and brood sex ratios in polyembryonic wasps. Nature 360:254.

Griffin, A. S., and S. A. West. 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 302:634–636.

Griffin, A. S., S. A. West, and A. Buckling. 2004. Cooperation and competition in pathogenic bacteria. Nature 430:1024.

Hamilton, W. D. 1964. The genetical theory of social evolution, I and II. J. Theoret. Biol. 7:1–52.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477–488.

Hamilton, W. D. 1970. Selfish and spiteful behaviour in an evolutionary model. Nature 228:1218.

Hamilton, W. D. 1971. Geometry for the selfish herd. J. Theoret. Biol. 31:295–311.

Hamilton, W. D. 1975. Innate social aptitudes of man: an approach from evolutionary genetics. Biosocial Anthropol. 53:133–55.

Harvey, P. H., and A. Purvis. 1991. Comparative methods for explaining adaptations. Nature 351:619.

Hatchwell, B. J. 2009. The evolution of cooperative breeding in birds: kinship, dispersal and life history. Philos. Trans. R Soc. B Biol. Sci. 364:3217–3227.

Hughes, W. O., B. P. Oldroyd, M. Beekman, and F. L. Ratnieks. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213–1216.

Inglis, R. F., A. Gardner, P. Cornelis, and A. Buckling. 2009. Spite and virulence in the bacterium *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. 106:5703–5707.

Innocent, T. M., J. Abe, S. A. West, and S. E. Reece. 2010. Competition between relatives and the evolution of dispersal in a parasitoid wasp. J. Evol. Biol. 23:1374–1385.

Irwin, A. J., and P. D. Taylor. 2001. Evolution of altruism in stepping-stone populations with overlapping generations. Theoret. Popul. Biol. 60:315–325.

Johnstone, R. A., and M. A. Cant. 2008. Sex differences in dispersal and the evolution of helping and harming. The American Naturalist 172:318–330.

Johnstone, R. A., and M. A. Cant. 2010. The evolution of menopause in cetaceans and humans: the role of demography. Proc. R Soc. Lond. B Biol. Sci.

Johnstone, R. A. 2008. Kin selection, local competition, and reproductive skew. Evolution 62:2592–2599.

Kell, D. B., A. S. Kaprelyants, and A. Grafen. 1995. Pheromones, social behaviour and the functions of secondary metabolism in bacteria. Trends Ecol. Evol. 10:126–129.

Kelly, J. K. 1992. Kin selection in density regulated populations. J. Theoret. Biol. 157:447–461.

Komdeur, J. 1994. The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). Proc. R Soc. Lond. B 256:47–52.

Komdeur, J., D. S. Richardson, and T. Burke. 2004. Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. Proc. R Soc B Biol. Sci. 271:963.

Kümmerli, R., A. Gardner, S. A. West, and A. S. Griffin. 2009. Limited dispersal, budding dispersal, and cooperation: an experimental study. Evolution 63:939–949.

Kuzdzal-Fick, J. J., S. A. Fox, J. E. Strassmann, and D. C. Queller. 2011. High relatedness is necessary and sufficient to maintain multicellularity in *Dictyostelium*. Science 334:1548–1551.

Lehmann, L., K. Bargum, and M. Reuter. 2006. An evolutionary analysis of the relationship between spite and altruism. J. Evol. Biol. 19:1507–1516.

Lehmann, L., and F. Rousset. 2010. How life history and demography promote or inhibit the evolution of helping behaviours. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:2599–2617.

Lehmann, L., N. Perrin, and F. Rousset. 2006. Population demography and the evolution of helping behaviors. Evolution 60:1137–1151.

Lehmann, L., L. Keller, S. West, and D. Roze. 2007. Group selection and kin selection: two concepts but one process. Proc. Natl. Acad. Sci. 104:6736–6739.

Lehmann, L., V. Ravigné, and L. Keller. 2008. Population viscosity can promote the evolution of altruistic sterile helpers and eusociality. Proc. R Soc. Lond. B Biol. Sci. 275:1887–1895.

Levin, S. R., and S. A. West. 2017a. Kin selection in the RNA world. Life 7:53.

Levin, S. R., and S. A. West. 2017b. The evolution of cooperation in simple molecular replicators. Proc. R Soc. B 284:20171967.

Liao, X., S. Rong, and D. C. Queller. 2015. Relatedness, conflict, and the evolution of eusociality. PLoS Biol. 13:e1002098.

Lion, S. 2013. Multiple infections, kin selection and the evolutionary epidemiology of parasite traits. J. Evol. Biol. 26:2107–2122.

Lion, S., and S. Gandon. 2009. Habitat saturation and the spatial evolutionary ecology of altruism. J. Evol. Biol. 22:1487–1502.

Lion, S., V. A. Jansen and T. Day. 2011. Evolution in structured populations: beyond the kin versus group debate. Trends in Ecology & Evolution 26:193–201.

Lukas, D., and T. Clutton-Brock. 2012a. Cooperative breeding and monogamy in mammalian societies. Proc. R Soc. Lond. B Biol. Sci. 279:2151–2156.

Lukas, D., and T. Clutton-Brock. 2012b. Life histories and the evolution of cooperative breeding in mammals. Proc. R Soc. Lond. B Biol. Sci. 279:4065–4070.

May, R. M. 2004. Uses and abuses of mathematics in biology. Science 303:790–793.

Mehdiabadi, N. J., C. N. Jack, T. T. Farnham, T. G. Platt, S. E. Kalla, G. Shaulsky, et al. 2006. Social evolution: kin preference in a social microbe. Nature 442:881.

Mitteldorf, J., and D. S. Wilson. 2000. Population viscosity and the evolution of altruism. J. Theoret. Biol. 204:481–496.

Nee, S., S. A. West, and A. F. Read. 2002. Inbreeding and parasite sex ratios. Proc. R Soc. Lond. B Biol. Sci. 269:755–760.

Noh, S., K. S. Geist, X. Tian, J. E. Strassmann, and D. C. Queller. 2018. Genetic signatures of microbial altruism and cheating in social amoebas in the wild. Proceedings of the National Academy of Sciences 201720324.

Ohtsuki, H., C. Hauert, E. Lieberman, and M. A. Nowak. 2006. A simple rule for the evolution of cooperation on graphs and social networks. Nature 441:502.

Ostrowski, E. A., Y. Shen, X. Tian, R. Sucgang, H. Jiang, J. Qu, et al. 2015. Genomic signatures of cooperation and conflict in the social amoeba. Curr. Biol. 25:1661–1665.

Parker, G. A., and J. Maynard Smith. 1990. Optimality theory in evolutionary biology. Nature 348:27.

Pen, I., and F. J. Weissing. 2000. Towards a unified theory of cooperative breeding: the role of ecology and life history re-examined. Proc. R Soc. Lond. B Biol. Sci. 267:2411–2418.

Peña, J., G. Nöldeke, and L. Lehmann. 2015. Evolutionary dynamics of collective action in spatially structured populations. J. Theoret. Biol. 382:122–136.

Perrin, N., and L. Lehmann. 2001. Is sociality driven by the costs of dispersal or the benefits of philopatry? A role for kin-discrimination mechanisms. Am. Nat. 158:471–483.

Pfennig, D. W., and J. P. Collins. 1993. Kinship affects morphogenesis in cannibalistic salamanders. Nature 362:836.

Pfennig, D. W., J. P. Collins, and R. E. Ziemba. 1999. A test of alternative hypotheses for kin recognition in cannibalistic tiger salamanders. Behav. Ecol. 10:436–443.

Pfennig, D. W., P. W. Sherman, and J. P. Collins. 1994. Kin recognition and cannibalism in polyphenic salamanders. Behav. Ecol. 5:225–232.

Pollitt, E. J., S. A. West, S. A. Crusz, M. N. Burton-Chellew, and S. P. Diggle. 2014. Cooperation, quorum sensing, and evolution of virulence in *Staphylococcus aureus*. Infection Immunity 82:1045–1051.

Popat, R., E. J. Pollitt, F. Harrison, H. Naghra, K. W. Hong, K. G. Chan, A. S. Griffin, P. Williams, S. P. Brown, S. A. West and S. P. Diggle. 2015. Conflict of interest and signal interference lead to the breakdown of honest signaling. Evolution 69:2371–2383.

Queller, D. C. 1994. Genetic relatedness in viscous populations. Evol. Ecol. 870–873.

Ratnieks, F. L. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am. Nat. 132:217–236.

Ratnieks, F. L., K. R. Foster, and T. Wenseleers. 2006. Conflict resolution in insect societies. Annu. Rev. Entomol. 51:581–608.

Rautiala, P., H. Helanterä, and M. Puurtinen. 2014. Unmatedness promotes the evolution of helping more in diplodiploids than in haplodiploids. Am. Nat. 184:318–325.

Rodrigues, A. M., and A. Gardner. 2012. Evolution of helping and harming in heterogeneous populations. Evolution 66:2065–2079.

Rodrigues, A. M., and A. Gardner. 2013a. Evolution of helping and harming in heterogeneous groups. Evolution 67:2284–2298.

Rodrigues, A. M., and A. Gardner. 2013b. Evolution of helping and harming in viscous populations when group size varies. Am. Nat. 181:609–622.

Rogers, A. R. 1990. Group selection by selective emigration: the effects of migration and kin structure. Am. Nat. 135:398–413.

Rousset, F. 2004. Genetic structure and selection in subdivided populations (MPB-40). Princeton Univ. Press, Princeton.

Rumbaugh, K. P., U. Trivedi, C. Watters, M. N. Burton-Chellew, S. P. Diggle, and S. A. West. 2012. Kin selection, quorum sensing and virulence in pathogenic bacteria. Proc. R. Soc. B 279:3584–3588.

Russell, A. F., and B. J. Hatchwell. 2001. Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. Proc. R Soc. Lond. B Biol. Sci. 268:2169–2174.

Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. Quart. Rev. Biol. 79:135–160.

Sharp, S. P., A. McGowan, M. J. Wood, and B. J. Hatchwell. 2005. Learned kin recognition cues in a social bird. Nature 434:1127.

Taylor, P. D., and S. A. Frank. 1996. How to make a kin selection model. J. Theoret. Biol. 180:27–37.

Taylor, P. D., and A. J. Irwin. 2000. Overlapping generations can promote altruistic behavior. Evolution 54:1135–1141.

Taylor, P. D. 1988. An inclusive fitness model for dispersal of offspring. J. Theoret. Biol. 130:363–378.

Taylor, P. D. 1992a. Altruism in viscous populations–an inclusive fitness model. Evolutionary Ecology 6:352–356.

Taylor, P. D. 1992b. Inclusive fitness in a homogeneous environment. Proc. R Soc. Lond. B 249:299–302.

Taylor, P. D., T. Day, and G. Wild. 2007. Evolution of cooperation in a finite homogeneous graph. Nature 447:469.

Traulsen, A., and M. A. Nowak. 2006. Evolution of cooperation by multilevel selection. Proceedings of the National Academy of Sciences 103:10952–10955.

Trivers, R. L., and H. Hare. 1976. Haploidploidy and the evolution of the social insect. Science 191:249–263.

Van Baalen, M., and D. A. Rand. 1998. The unit of selection in viscous populations and the evolution of altruism. J. Theoret. Biol. 193:631–648.

Van Cleve, J. 2015. Social evolution and genetic interactions in the short and long term. Theoret. Popul. Biol. 103:2–26.

Van Cleve, J., and L. Lehmann. 2013. Stochastic stability and the evolution of coordination in spatially structured populations. Theoret. Popul. Biol. 89:75–87.

Wenseleers, T., and F. L. Ratnieks. 2004. Tragedy of the commons in Melipona bees. Proceedings of the Royal Society of London B: Biological Sciences 271(Suppl 5):S310–S312.

Wenseleers, T., and F. L. Ratnieks. 2006a. Comparative analysis of worker reproduction and policing in eusocial Hymenoptera supports relatedness theory. Am. Nat. 168:E163–E179.

Wenseleers, T., and F. L. Ratnieks. 2006b. Enforced altruism in insect societies. Nature 444:50.

Wenseleers, T., A. G. Hart, and F. L. Ratnieks. 2004a. When resistance is useless: policing and the evolution of reproductive acquiescence in insect societies. Am. Nat. 164:E154–E167.

Wenseleers, T., H. Helanterä, A. Hart, and F. L. Ratnieks. 2004b. Worker reproduction and policing in insect societies: an ESS analysis. J. Evol. Biol. 17:1035–1047.

Wenseleers, T., F. L. Ratnieks, and J. Billen. 2003. Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis. J. Evol. Biol. 16:647–658.

West, S. A., and A. Buckling. 2003. Cooperation, virulence and siderophore production in bacterial parasites. Proc. R Soc. Lond. B Biol. Sci. 270:37–44.

West, S. A., A. S. Griffin, and A. Gardner. 2007a. Evolutionary explanations for cooperation. Curr. Biol. 17:R661–R672.

West, S. A., A. S. Griffin, and A. Gardner. 2007b. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. J. Evol. Biol. 20:415–432.

West, S. A., E. T. Kiers, E. L. Simms, and R. F. Denison. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? Proc. R Soc. Lond. B Biol. Sci. 269:685–694.

West, S. A., M. G. Murray, C. A. Machado, A. S. Griffin, and E. A. Herre. 2001. Testing Hamilton's rule with competition between relatives. Nature 409:510.

West, S. 2009. Sex allocation. Princeton Univ. Press, Princeton.

Westneat, D., and C. W. Fox (Eds.). 2010. Evolutionary behavioral ecology. Oxford Univ. Press, Oxford.

Wild, G., and S. A. West. 2007. A sex allocation theory for vertebrates: combining local resource competition and condition-dependent allocation. Am. Nat. 170:E112–E128.

Wild, G., A. Gardner, and S. A. West. 2009. Adaptation and the evolution of parasite virulence in a connected world. Nature 459:983.

Wild, G. 2011. Direct fitness for dynamic kin selection. J. Evol. Biol. 24:1598–1610.

Yeh, A. Y. C., and A. Gardner. 2012. A general ploidy model for the evolution of helping in viscous populations. J. Theoret. Biol. 304:297–303.

Associate Editor: A. Gardner

Supplementary materials 5. Cheating and resistance to cheating in natural populations of the bacterium *Pseudomonas fluorescens* (published paper)

doi:10.1111/evo.13328

Cheating and resistance to cheating in natural populations of the bacterium *Pseudomonas fluorescens*

John B. Bruce,[1,2] Guy A. Cooper,[1] Hélène Chabas,[3] Stuart A. West,[1] and Ashleigh S. Griffin[1]

[1]*Department of Zoology, University of Oxford, Oxford, UK*

[2]*E-mail: john.bruce@zoo.ox.ac.uk*

[3]*CEFE UMR 5175, CNRS-Université de Montpellier, Université Paul-Valéry Montpellier, Montpellier Cedex 5, France*

Received October 26, 2016
Accepted August 8, 2017

Bacteria perform cooperative behaviors that are exploitable by noncooperative cheats, and cheats frequently arise and coexist with cooperators in laboratory microcosms. However, evidence of competitive dynamics between cooperators and cheats in nature remains limited. Using the production of pyoverdine, an iron-scavenging molecule, and natural soil populations of *Pseudomonas fluorescens*, we found that (1) nonproducers are present in the population; (2) they co-occur (<1cm^3) with pyoverdine producers; (3) they retain functional pyoverdine receptors; and (4) they can use the pyoverdine of on average 52% of producers. This suggests nonproducers can potentially act as social cheats in soil: utilizing the pyoverdine of others while producing little or none themselves. However, we found considerable variation in the extent to which nonproducers can exploit producers, as some isolates appear to produce exclusive forms of pyoverdine or kill nonproducers with toxins. We examined the consequences of this variation using theoretical modeling. We found variance in exploitability leads to some cheats gaining increased fitness benefits and others decreased benefits. However, the absolute gain in fitness from high exploitation is lower than the drop in fitness from low exploitation, decreasing the mean fitness of cheats and subsequently lowering the proportion of cheats maintained in the population. Our results suggest that although cooperator-cheat dynamics can occur in soil, a range of mechanisms can prevent nonproducers from exploiting producers.

KEY WORDS: **Bacteria, Cooperation, Cheating, Pyoverdine, Social Evolution, Soil.**

Cooperation is prevalent at all levels of biological organization: genes cooperate to produce cells, cells cooperate to produce organisms, and organisms cooperate to form societies (Bourke 2011). Yet cooperative behaviors are open to exploitation by noncooperative "cheats": individuals that increase their own fitness by exploiting the cooperative behaviors of others (Ghoul et al. 2014a). Theory suggests that a number of factors allow cheats and cooperators to coexist through frequency-dependent selection. Cheats can have an advantage when rare if this lets them better exploit cooperators (Ross Gillespie et al. 2007). Conversely, cooperators have an advantage when rare if the behavior provides some direct benefit, if they gain preferential access to cooperative goods, or if populations are spatially structured (Gore et al. 2009; Frank 2010; Koschwanez et al. 2011).

However, there remains a lack of evidence that cooperators and cheats coexist in natural settings (Harrison 2013; Jones et al. 2015; Riehl & Frederickson 2016). First, many studies examining cooperator-cheat dynamics make use of artificially created cheats (Greig & Travisano 2004; Griffin et al. 2004; Diggle et al. 2007; Sandoz et al. 2007;Gore et al. 2009). The extent to which similar cheats arise in natural populations remains unclear (Sachs et al. 2010; Bozdag & Greig 2014; Jones et al. 2015). Second, while less cooperative or noncooperative individuals are found in natural populations, this may reflect environments where there is selection for less cooperation, rather than cheating per se (Ghoul et al. 2014a,b; Jones et al. 2015). Third, there is a range of mechanisms that could prevent potential cheats from successfully exploiting the cooperative behavior of others (West et al. 2007). These

include preferentially directing cooperation toward relatives, and directing harming behaviors toward nonrelatives or noncooperators (Griffin & West 2003; Kiers et al. 2003; Sharp et al. 2005). For example, bacteria produce bacteriocins (toxins) that could potentially prevent cheats from exploiting cooperative behaviors (Riley & Gordon 1999; Wang et al. 2015).

We examine the extent to which cheating and the above complexities occur in natural populations of the soil bacterium *P. fluorescens*. The cooperative trait we examine is the production of an extracellular iron-scavenging molecule, pyoverdine (Varma & Chincholkar 2007). While iron is essential for bacterial growth, levels of soluble iron in soils are often low (Wandersmann & Delepelaire 2004, Lindsay & Schwab 1982, Colombo et al. 2014). Iron-starved cells secrete pyoverdine into the environment where it binds Fe(III) and is transported into the cell where Fe(III) is dissociated and reduced to bioavailable Fe(II) (Greenwald et al. 2007). However, after binding iron, pyoverdine molecules can in principle be taken up by any other cells with an appropriate receptor, not just the cell that produced them (West & Buckling 2003). Pyoverdine production has been studied extensively in the laboratory, where it has been demonstrated to be a cooperative trait open to exploitation by nonproducing cheats (Griffin et al. 2004; Jiricny et al. 2010; Ghoul et al. 2014b; Kummerli & Ross Gillespie 2013).

We examined isolates of *P. fluorescens* from soil at multiple sites in a local park and asked: (1) Do pyoverdine nonproducers exist in natural soil populations? (2) Can nonproducers exploit the pyoverdine produced by other cells in the population? One reason they might not be able to is if different strains produce different types of pyoverdine that require a specific receptor—this specificity could be thought of as a form of kin discrimination. (3) Do the bacteriocins produced by cells play a role in preventing pyoverdine exploitation? Bacteriocins tend to kill unrelated isolates, and so could be one mechanism of preventing unrelated lineages from exploiting cooperative behaviors (Inglis et al. 2009; Strassmann & Queller 2014). Finally, our experimental work revealed that nonproducers could exploit pyoverdine produced by some isolates and not others. Consequently, we used theoretical modeling to ask: (4) How does variation in the ability of cheats to exploit cooperators influence whether cheats and cooperators can coexist in the same population?

Material and Methods

SITE DESCRIPTION AND SAMPLING

We collected isolates of the *P. fluorescens* group from soil samples at multiple sites in University Parks, Oxford, as described previously (Bruce et al. 2017). The soil in the park is an alluvial sandy loam with a pH of ~7.8. Briefly, soil samples were collected from eight sites in undisturbed regions of the park and at each site we sampled a 1 m transect consisting of four patches: an initial patch (patch 0) and patches 1 cm (patch 1), 10 cm (patch 2), and 100 cm (patch 3) from the initial patch. We randomly selected seven isolates from each patch in each site, giving 224 isolates from 32 patches for use in the study. All isolates were sampled, cultured, isolated, and frozen in under 48 h to minimize the potential for evolution in the laboratory and all subsequent tests were performed using these freezer stocks.

PYOVERDINE SCREEN OF ISOLATES

We screened all isolates in the collection for the ability to produce pyoverdine, allowing us to identify low/nonproducing isolates and reveal any variation in pyoverdine production across the population. To do this, we cultured isolates from freezer stocks in 2 mL of Kings B (KB) [20 g protease peptone No3 (Beckton Dickinson Ltd., Oxford, UK), 10 mL glycerol, 1.5 g $K_2HPO_4.3H_2O$ and 1.5 g $MgSO_4.7H_2O$ per litre of dH_2O] media in 24-well plates at 25 °C, shaken overnight at 200 rpm. Cell density was standardized and diluted to an optical density of 0.2 (A_{600}) with M9 minimal media (6.8 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl and 10 g NH_4Cl per litre of dH_2O) and inoculated into 96-well plates containing iron-limited casamino acid media [5 g casamino acids, 1.18 g $K_2HPO_4.3H_2O$, 0.25 g $MgSO_4.7H_2O$, per litre of dH_2O supplemented with the iron chelator, human apo-transferrin 100 μg mL-1 (Sigma) and 20 mM $NaHCO_3$] (six replicates per isolate, 200 μL of media, and 2 μL of bacterial culture). We then incubated cultures for 48 h at 25 °C before measuring cell density (A_{600}) and fluorescence (400/460 nm excitation/emission, cutoff at 475 nm) using a fluorimeter (SpectraMax M2, Molecular Devices, California, USA). Pyoverdine production per cell was calculated as the relative fluorescent units (RFU) of a sample corrected for cell density. Isolates that grew poorly in iron-limited media and produced very little or no pyoverdine (those isolates with average values below the fifth percentile for pyoverdine production per cell) we classified as nonproducers. We also cultured isolates that we classified as nonproducers in iron-replete CAA media under the same conditions, to confirm that iron limitation and a failure to produce sufficient pyoverdine are responsible for poor growth in iron-limited media.

MLST OF ISOLATES

We used multilocus sequence typing (MLST) of three housekeeping genes to provide a measure of genetic similarity between isolates; allowing us to explore the relationship between successful cross-feeding and genetic similarity, and also the phylogenetic origins of any pyoverdine nonproducing isolates.

DNA extraction and PCR

We extracted genomic DNA using the Wizard Genomic DNA purification kit (Promega, Wisconsin, USA) and amplified three

housekeeping genes (gyrB, RpoB, and RpoD; Table S1.) for each isolate. All reactions were performed in 50 μL volume containing 1 U of DreamTaq polymerase (Thermo Fisher Scientific, Massachusetts, USA), 5 μL of DreamTaq Buffer, 100 mM of each primer, 0.2 mM of each dNTP, 40.75 μL of ddH_2O, and 20 ng of DNA template. We used the following cycling conditions: 95 °C for 3 min, then 35 cycles of 95 °C for 30 sec, 58 °C/60 °C/64.5 °C for 30 sec, 72 °C for 45 sec, and a final extension of 72 °C for 5 min (annealing temperatures; Table S1).

Sequencing and analysis

We purified PCR products before they were Sanger sequenced by SourceBioscience (Nottingham, U.K.) with the respective primer pairs used for PCR amplification used as forward and reverse sequencing primers (Table S1). The quality of the resulting sequences was checked using Geneious Pro (Biomatters Ltd, Auckland, New Zealand) generating a consensus sequence for each isolate before trimming and aligning the sequences to obtain an identical length sequence for each gene in each isolate. We then constructed a concatemer of all three genes for each isolate; from which we calculated pairwise genetic distances between isolate pairs using the Jukes-Cantor model and constructed a neighbor-joining tree using MEGA6 (Tamura et al. 2013).

TEST OF RECEPTOR FUNCTION

We tested isolates that we had identified as pyoverdine nonproducers for their ability to use purified pyoverdine to sequester iron from the environment, allowing us to assess their potential to act as cheats. Pyoverdine nonproducer cells are completely incapable of growth in iron-limited media supplemented with purified pyoverdine if they lack appropriate pyoverdine receptors (Ghysels et al. 2004). To do this, we cultured nonproducers in 2 mL of KB media in a 24-well plate at 25 °C, shaken overnight at 200 rpm. Cell density was standardized and diluted to an optical density of 0.2 (A_{600}) using M9 minimal media before inoculating 2 μL into 96-well plates containing 200 μL of iron-limited media (12 replicates). Half of each isolates replicates were supplemented with purified pyoverdines from *P. fluorescens* (Sigma, Missouri, USA) to approximately 800 RFU and wells containing supplemented and nonsupplemented iron-limited media, but free of cells, served as negative controls. We incubated cultures at 25 °C for 24 h and then measured cell density (A_{600}) in supplemented and unsupplemented replicates.

PYOVERDINE CROSS-FEEDING EXPERIMENTS

We tested nonproducing isolates for their ability to use the pyoverdine-containing supernatant of other isolates to increase their growth, allowing us to determine whether nonproducers are potentially able to exploit pyoverdine producers in the population. First, we extracted supernatant from cultures of pyoverdine producing isolates. Pyoverdine producers were cultured in 2 mL of KB media in 24-well plates at 25 °C, shaken overnight at 200 rpm, and standardized cell density to an optical density of 0.2 (A_{600}) using M9 minimal medium before inoculating 60 μL into 6 mL of iron-limited CAA in 30-mL glass vials. Cultures were incubated statically for 48 h at 25 °C before being passed through a 0.22 μm filter to remove bacterial cells. We passed the cell-free supernatants through a centrifugal unit containing a 3 kDa membrane (EMD Millipore, Massachusetts, USA) to remove cellular debris and large extracellular products, such as bacteriocins, while retaining pyoverdine molecules (Cordero et al. 2012). Purified supernatants were stored at −20 °C until required for growth enhancement experiments.

We then assayed for the effects of the supernatant extracted as described above, on growth of nonproducing isolates. Nonproducers were cultured in 2 mL of KB media in 24-well plates at 25 °C, shaken overnight at 200 rpm before standardizing cell density to an optical density of 0.2 (A_{600}) with M9 minimal media. Two microliters of standardized culture was inoculated into 96-well plates containing 180 μL of iron-limited media which we supplemented with 20 μL of purified supernatant from a pyoverdine-producing isolate. Each nonproducer (11 isolates) was grown in iron-limited CAA media supplemented with the supernatant of each isolate in the collection (224 growth enhancement tests per nonproducer, five replicates each) and alone in 200 μL of iron-limited CAA media. We incubated cultures for 24 h at 25 °C before measuring final cell density at A_{600}.

ASSESSING NONPRODUCER SUSCEPTIBILITY TO PRODUCERS BACTERIOCINS

Competitive behaviors that harm or kill competitors may potentially protect pyoverdine producers from exploitation. We assayed whether potential cheats in our study were more likely to be inhibited by the bacteriocins of producing strains they were able to exploit, compared to those they were unable to exploit. First, we extracted bacteriocin-containing supernatants from each isolate in the population by culturing isolates for 24 h in 6 mL of KB media (23 °C at 200 rpm), measuring cell density and standardized each culture to an optical density of ∼0.3 (A_{600}). These cultures were then diluted 10-fold in fresh KB media and incubated shaking for 24 h at 23 °C. We then centrifuged cultures at 6861 rpm for 10 min, obtaining a clear, cell-free supernatant by filter sterilizing with a 0.22 μm filter and storing at −20 °C until required.

We then randomly selected 10 isolates that each pyoverdine nonproducer was capable of exploiting and 10 isolates each was incapable of exploiting and determined the ability of these isolates to inhibit each nonproducers growth with bacteriocins. We spotted KB agar plates with bacteriocin-containing supernatants, spread a lawn of each pyoverdine nonproducer over the plate,

and recorded which supernatants inhibited which nonproducer. Assays were carried out in triplicate. We spotted KB agar plates with 15 μL of supernatant and allowed the spots to dry at room temperature. We cultured pyoverdine nonproducers from freezer stocks for 24 h in 6 mL of KB media (23 °C at 200 rpm). Cell density was standardized to an optical density of ∼0.1 (A_{600}) and diluted 10-fold in M9 before 70 μL of culture was spread onto the supernatant-spotted KB agar plates. We incubated plates at 23 °C for ∼14 h, or until a uniform lawn of bacterial growth was visible, checked the plates for zones of inhibition on and around the supernatant spots and recorded whether a lawn was inhibited by a particular supernatant. Inhibition was recorded as a binary response (one for inhibition, zero for no inhibition).

STATISTICAL ANALYSIS

Of the 11 isolates we previously designated as nonproducers, we merged and averaged the data from isolates that were genetically identical at three housekeeping genes, resulting in eight independent samples. We wished to determine whether a nonproducer could use the pyoverdine of other producers in the population. To do this, we tested for significant differences between these eight nonproducers' growth in iron-limited media and in iron-limited media supplemented with the supernatants of pyoverdine producers using linear models, with final cell density as the response variable and supernatant as a categorical explanatory variable. If a nonproducer grew significantly better in supplemented iron-limited media than it did alone, we considered this successful use of the producers' pyoverdine. We recorded which supernatants nonproducers successfully used in a binary 8×224 matrix.

We carried out all statistical analyses in the R statistical environment. Except where stated, we carried out standard analyses (ANOVA, GLM, T-test etc.) assuming normal errors. All analyses using generalized linear mixed models (GLMM) included the identity of the nonproducer as a random effect, to account for the fact that we have multiple nonproducers in the population.

Results

VARIATION IN PYOVERDINE PRODUCTION

We identified 11 isolates (4.5% of the population) with values below the fifth percentile value for pyoverdine production per cell, fulfilling our criteria for consideration as nonproducers, and hence potential cheats (Fig. 1A). These isolates grow well under iron-replete conditions, indicating that the availability of iron limits the growth of nonproducers in this media (Fig. 1B). Across all isolates, growth in iron-limited media was highly correlated with pyoverdine production (LM: $t = 8.543$, $P = 2.19e^{-15}$).

Pyoverdine production per cell varied significantly both between patches (Fig. 1B, ANOVA: $F = 2.228$, $P = 0.0005$) and between transects (ANOVA: $F = 3.015$, $P = 0.00484$) in the population. Significant differences between patches and between transects appear to be driven by transect I: patch I3 had significantly higher average levels of pyoverdine production per cell than three other patches and transect I had significantly higher levels of pyoverdine production per cell than two other transects (TukeyHSD test, Table S3). Isolates previously designated as nonproducers always co-occurred with pyoverdine producers and were found at 8 of the 32 patches sampled. The neighbor-joining tree suggests that nonproduction has arisen at least six times in the population (Fig. 1B, 2A.).

72% of isolates ($n = 224$) in the collection were genetically distinct, with the pairwise genetic distance between isolates ranging from 0.000 to 0.066 and averaging 0.04.

NONPRODUCERS HAVE RETAINED RECEPTOR FUNCTION

Nonproducers grown in iron-limited media supplemented with purified pyoverdine all grow significantly better than when cultured in iron-limited media (Fig S1, Table S3), suggesting that isolates have retained functional pyoverdine receptors despite no longer producing pyoverdine.

CROSS-FEEDING ASSAYS REVEAL DIVERSE PATTERNS OF SUCCESSFUL AND UNSUCCESSFUL SUPERNATANT USE

The growth of nonproducers in iron-limited media significantly increased when supplemented with the supernatant of on average 52% of pyoverdine-producing isolates. However, the cross-feeding assays reveal diverse patterns of successful and unsuccessful use of producers' supernatant to increase growth (Fig. 2A, C). A minority of pyoverdine producers supernatants significantly increased the growth of all nonproducers (12%), some supernatants increased the growth of none of the nonproducers (23%) and the remainder (65%) of supernatants significantly increased the growth of some nonproducers but not others (Fig. 2B).

There are at least three possible explanations for this observation: (1) Although we have demonstrated that iron is a significant growth-limiting factor in this media (Fig. 1B), we have not demonstrated that iron is the *only* limiting factor: it may be possible that metabolites other than pyoverdine in the supernatant can increase or decrease the growth of nonproducers. It has also been suggested that siderophores may act as a trace metal buffers, increasing the availability of iron to nonproducers without requiring the appropriate receptor. However, pyoverdine nonproducer cells are incapable of growth in iron-limited media supplemented with purified pyoverdine if they lack appropriate pyoverdine receptors, suggesting pyoverdine does not act as a trace metal buffer (Ghysels et al. 2004). (2) The amount of pyoverdine in producers

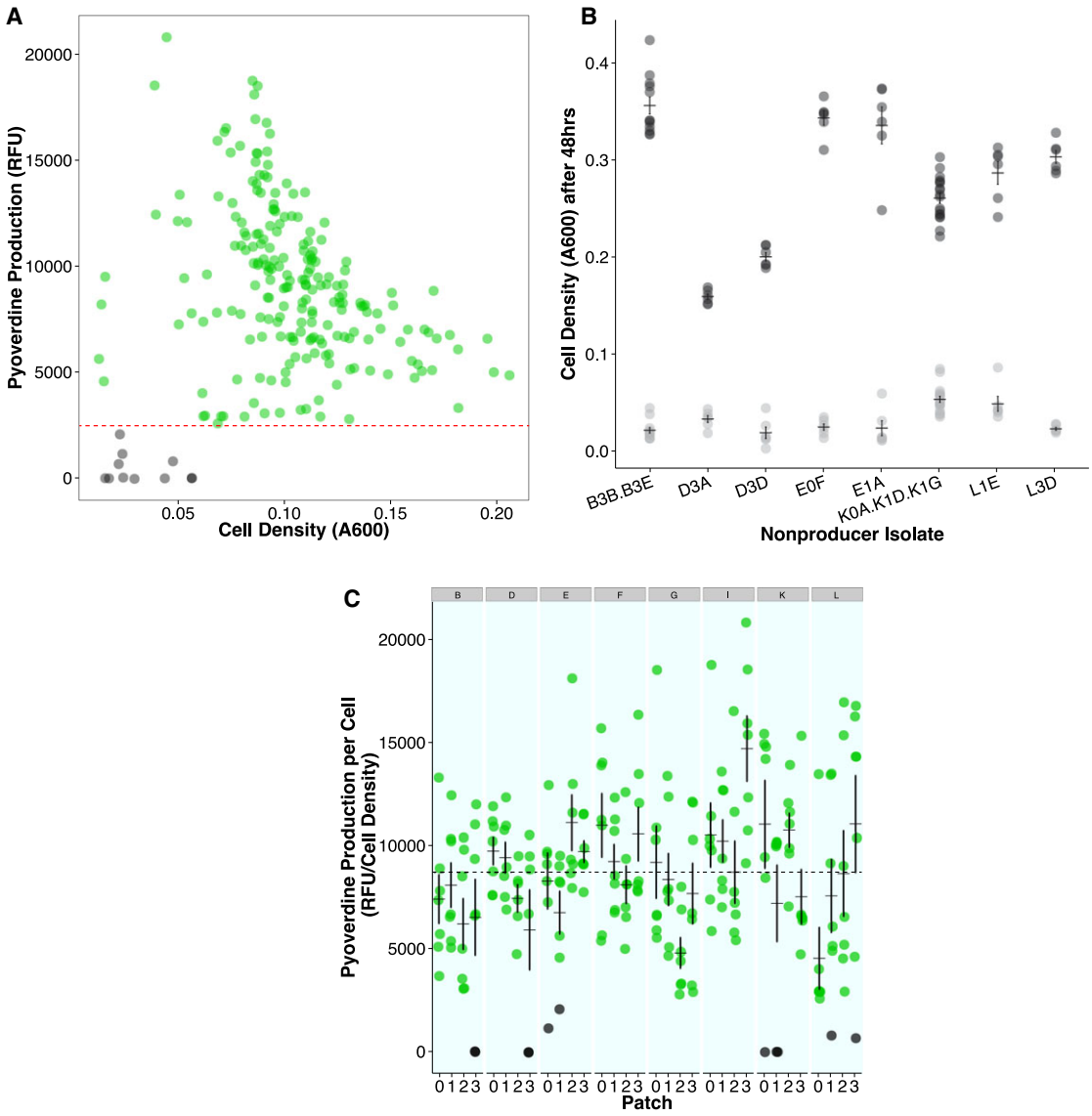


Figure 1. Pyoverdine production per cell and growth of isolates in iron-limited media. (A) Isolates falling below the fifth percentile for pyoverdine production per cell were considered non producers. The horizontal red dashed line represents the fifth percentile for pyoverdine production per cell. Gray circles represent isolates classified as nonproducers and green circles represent pyoverdine producers; values are the mean of six replicates. (B) Growth of nonproducer isolates in CAA media and in iron-limited CAA media. All nonproducer isolates grow well in CAA media but show significantly reduced growth in iron-limited CAA media. Light grey circles represent nonproducers growth in iron-limited CAA media and dark grey circles represent nonproducers growth in CAA media. (C) Pyoverdine production per cell varies significantly between patches and between transects, and nonproducers co-occur with pyoverdine producers. Gray circles represent isolates classified as nonproducers and green circles represent pyoverdine producers. Horizontal bars are average per cell production for each patch ± SE.

supernatants varies and this might explain variation in exploitability, that is, addition of supernatants resulting in no significant increase in growth might simply contain very little pyoverdine. However, we compared the levels of pyoverdine in producer supernatant for cross-feeding experiments that resulted in a significant growth increase versus no significant growth increase for nonproducers (Fig. S2), and found that the distributions do not significantly differ from each other (Kolmogorov-Smirnov test, $D = 0.1818$, P-value $= 0.9934$). This suggests that the amount of pyoverdine in the supernatant does not explain the variation in exploitability during cross-feeding assays. (3) The diverse patterns of successful and unsuccessful use of producer supernatants may occur because the population produces multiple forms of pyoverdine and these are not equally accessible to other isolates in the population. There is evidence that multiple different forms of pyoverdine are produced by *Pseudomonads* and that strains

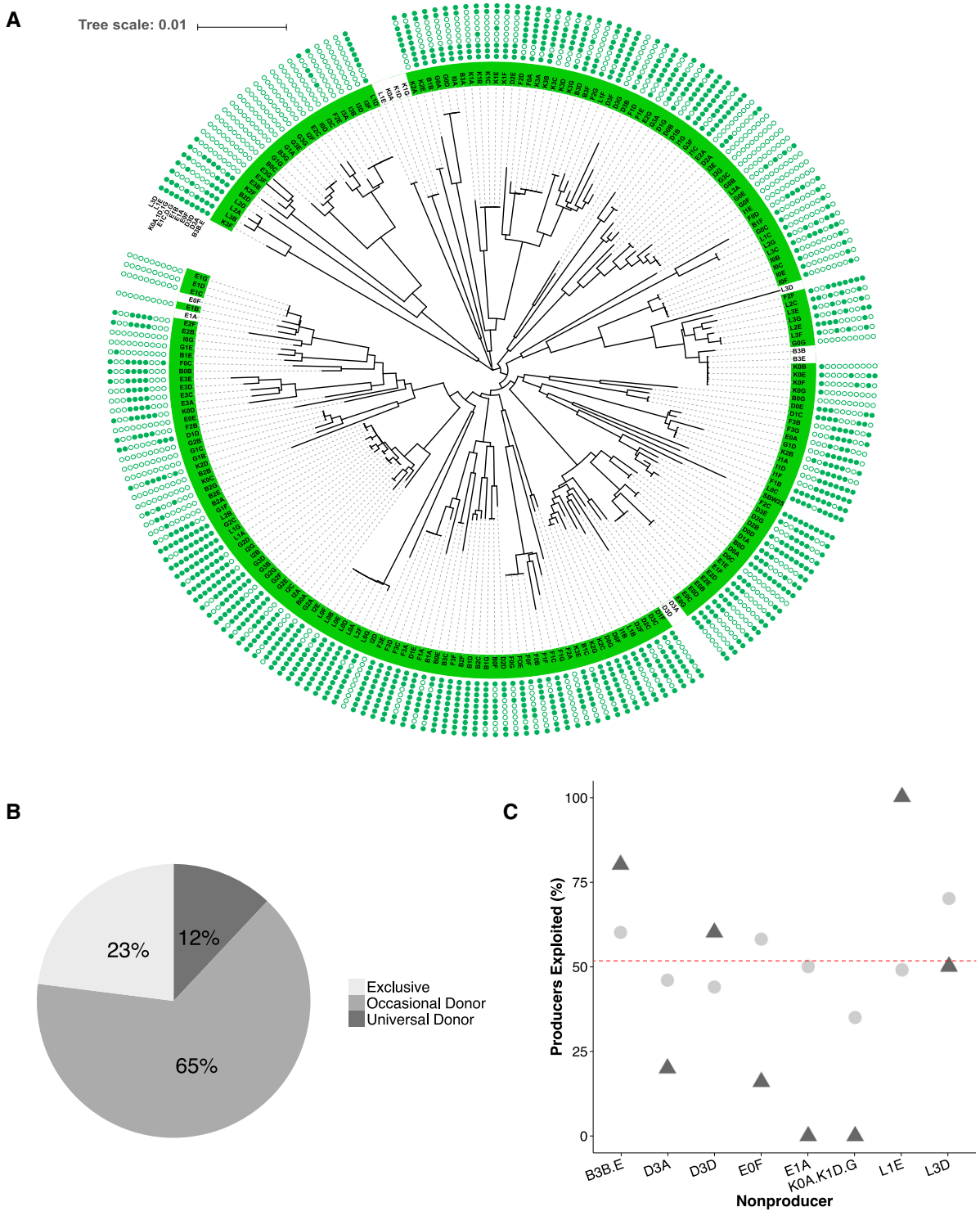


Figure 2. (A) Phylogenetic tree of all isolates in the population. The phylogenetic tree was constructed from a concatemer of three housekeeping genes (gyrB, recA, and rpoB) using the Neighbor-joining method and the Jukes-Cantor model of nucleotide substitution with 1000 bootstraps. Pyoverdine-producing isolates labels are highlighted in green, nonproducer labels are left blank. Each pyoverdine producing isolate has eight circles associated with it, each representing the ability of a specific nonproducer to use that isolates pyoverdine. Filled circles represent successful use of the isolates pyoverdine by the nonproducer in the cross-feeding assays and empty circles represent unsuccessful use. (B) Percentage of pyoverdine producers that act as universal donors, partial donors, and nondonors to pyoverdine nonproducers. (C) The percentage of producers in the population that nonproducers can exploit. Circles represent the percentage of isolates in the population that individual nonproducers can exploit, triangles represent the percentage of local, co-occuring isolates (from the same patch) that nonproducers can exploit.

vary in their ability to exploit the pyoverdine produced by others (Meyer 2000; Meyer et al. 2002).

GENETIC SIMILARITY PREDICTS ABILITY OF NONPRODUCERS TO USE PRODUCERS PYOVERDINES

We found that genetic similarity between nonproducers and producers predicted the ability of nonproducers to successfully use the producers' pyoverdine. Nonproducer isolates were more frequently able to use the pyoverdine of genetically similar producers than those of genetically more dissimilar isolates (Fig. 3A, GLM: $t = -2.846$, $P = 0.006$). The average genetic distance between nonproducers and co-occurring producers (from the same patch) was 0.038, ranging from 0.015 to 0.065.

NONPRODUCERS VARY IN THEIR ABILITY TO USE THE PYOVERDINE OF LOCAL PRODUCERS

Nonproducer isolates were no less likely to use the pyoverdine of co-occuring producers (from the same patch) than producers from the population as a whole (Fig. 3B, GLMM, $z = 1.529$, $P = 0.126$). Specifically, nonproducers could successfully use the pyoverdine of approximately 41.5% of co-occuring isolates and 52% of all other isolates in the population (Fig. 3B). However, the frequency of successful usage varies considerably between different nonproducers when interacting with co-occuring pyoverdine producers. This ranges from a complete inability of some nonproducers to use the pyoverdine of co-occuring producers, through to successful use of all surrounding producers pyoverdine by others. The variance in the frequency of exploitation was significantly greater when interactions occur between local, co-occurring producers and nonproducers (Fig. 3B, $F = 11.888$, df $= 7$, $P = 0.002075$).

PYOVERDINE PRODUCERS CAN INHIBIT NONPRODUCERS USING BACTERIOCINS

Overall, we found that in 3.5% of the interactions we tested between pyoverdine producers and nonproducers, the pyoverdine producers also released a bacteriocin that inhibited the nonproducer, and hence would have prevented the nonproducers from successfully cheating. The likelihood of a pyoverdine producer also releasing a bacteriocin that inhibited the nonproducer did not significantly vary dependent upon whether the nonproducer could use the pyoverdine from that producer (GLMM, $z = 0.452$, $P = 0.651$).

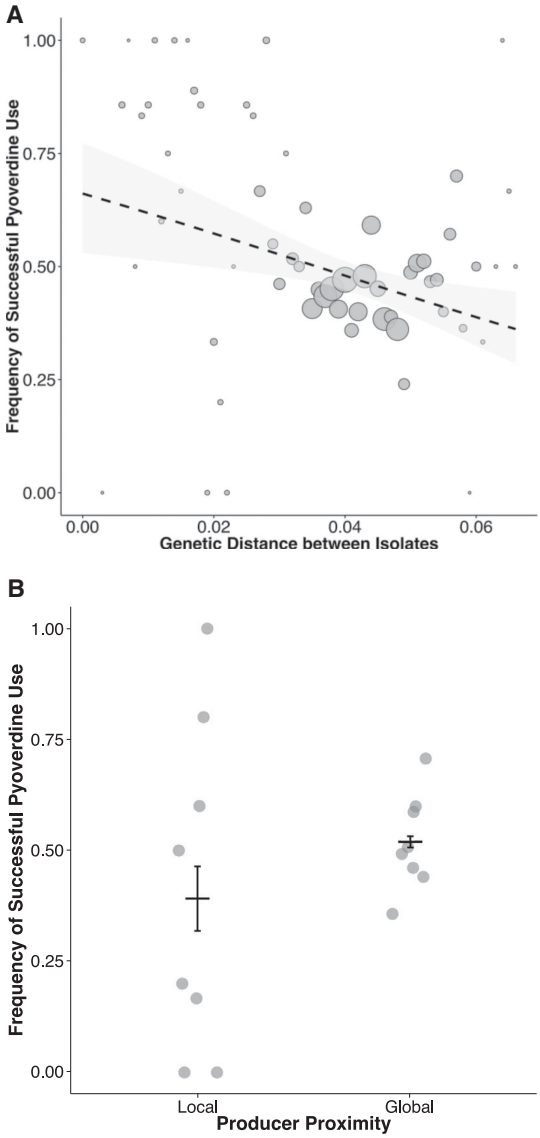


Figure 3. **The effects of genetic similarity and proximity on ability to use pyoverdine. (A) Nonproducer isolates can successfully use the pyoverdine of genetically similar isolates more frequently than genetically dissimilar isolates. The size of the points reflects the number of interactions: smallest points indicate <5 interactions and largest points indicate >150 interactions ($n = 1802$). (B) Nonproducer isolates are no less likely to use the pyoverdine of co-occuring producers than from producers from the rest of the population. Points reflect the proportion of successful pyoverdine use for each nonproducer when grown with co-occuring isolates (local) or all other isolates in the population (global). Horizontal bars are average proportion of successful pyoverdine use ± SE.**

EXPLOITABILITY AND CHEAT-COOPERATOR COEXISTENCE

We found that there is significant variation in the extent to which potential cheats can exploit cooperators, which is caused by variation in both pyoverdine exploitability and bacteriocin production (Fig. 2A–C). In this section, we examined theoretically the influence of such variation for whether cheats and cooperators will coexist. Our hypothesis is that, because the benefits from cooperation are often diminishing (Ross-Gillespie et al. 2007; Gore et al. 2009; Cornforth et al. 2012, Frank 2011), variance

in exploitability will decrease the relative fitness of cheats, and hence make it harder for them to coexist with cooperators.

We model a simple public goods scenario that could be applicable to a range of microbial traits (West et al. 2007). We assume a large population of cells composed of cooperators and cheats. The cooperators invest a fraction $q \in [0, 1]$ of their resources into the production of an extracellular factor that provides a benefit to the local population of cells (public good). We assume that the benefit from a fraction $1 - \lambda$ of the dispersed good is returned to the cell that produces it, and that the remaining fraction λ goes to other cells. The parameter λ determines how well the good disperses and is shared—a lower value of λ implies less shared and, in the extreme of $\lambda = 0$, we have a private good. If bacteria gain a greater than random share of any extracellular factor that they produce, this can lead to the fitness of cheats being frequency dependent (Ross-Gillespie et al. 2007; Gore et al. 2009). In contrast, the cheats do not produce the extracellular factor. We assume that the population is composed of a fraction x of cooperators and a fraction $1 - x$ of cheats.

We allow the benefit obtained from the extracellular factor to be nonlinear, as determined by the shape parameter α (>0) (Fig. 4A). We assume $\alpha < 1$, such that the synergistic effect is diminishing, as is thought to be the case for extracellular factors such as iron scavenging siderophore molecules (Ross-Gillespie et al. 2007). This gives the following personal fitness for a focal cooperator cell:

$$W_C = (1 - q) \times ((1 - \lambda) q + x\lambda Q)^{\alpha}, \quad (1)$$

where Q is the average production of the dispersed good by other cooperator cells (others-only; Pepper 2000). The first term quantifies the private cost due to production of the good $(1 - q)$ and the second term captures the benefit from the dispersed good (produced by the focal cell, and other cells).

We assume that there is variance in how well the cheats can exploit the extracellular factor produced by different strains of cooperators. For simplicity, we assume that cheats can find themselves in two scenarios: with probability p, they are relatively good at exploiting the extracellular factor in their local environment $(E_0 + \Delta E)$, and with probability $1 - p$, they are relatively bad at exploiting the extracellular factor in their local environment $(E_0 - \Delta E)$. We assume that each environment is equally likely $(p = 1/2)$. Thus, the parameter E_0 is the expected exploitation of a cheat and ΔE quantifies the variance in cheater exploitation, which will be high if there is a large difference in how well cheats can exploit some cooperative strains over others. This leads to the following personal fitness of a focal cheater cell:

$$W_D = p(x\lambda Q(E_0 - \Delta E))^{\alpha} + p(x\lambda Q(E_0 + \Delta E))^{\alpha}, \quad (2)$$

where the first and second terms are the realized fitness in the low and high exploitation environments, respectively. We assume that cooperators throughout the population are monomorphic with respect to cooperation and thus that $Q = q$, which we hold fixed in this analysis.

As the proportion of cheats in the population increases, the proportion of cooperators necessarily decreases and, as a result, the background density of the dispersed good drops for both cheats and cooperators (Fig. 4B). Cheater fitness therefore increases as cheaters become more rare and decreases as cheats become more common in the population (frequency dependence; Ross-Gillespie et al. 2007; Gore et al. 2009).

We ask how the variation in the extent to which cheats can exploit cooperators (ΔE) influences the equilibrium proportion of cheats ($1 - x^*$). We set cooperator and cheat fitness as equal and solve for x^*, giving:

$$1 - x^* = \max\left(\frac{\lambda\theta p^{1/\alpha} - (1 - q)^{1/\alpha}}{\lambda\left(\theta p^{1/\alpha} - (1 - q)^{1/\alpha}\right)}, 0\right), \quad (3)$$

where $\theta = ((E_0 - \Delta E)^{\alpha} + (E_0 + \Delta E)^{\alpha})^{1/\alpha}$.

Discussion

Our results suggest that in natural bacterial populations, pyoverdine nonproducers can potentially act as social cheats. We found that 4.5% of all isolates in the population do not produce pyoverdine, and these nonproducers were found in over a quarter (28%) of the 32 patches sampled (Fig. 1A, C). However, nonproducers can exploit on average only 52% of pyoverdine producers in the population and only 41.5% of local, co-occuring producers (Fig. 2A, C). Our cross-feeding assays suggest different forms of pyoverdine are produced in the population (Fig. 2A, B). In 3.5% of interactions, pyoverdine producers produced toxins that killed nonproducers, contributing to variation in the extent to which nonproducers can exploit producers. Furthermore, our model suggests that this variability in exploitability may reduce the mean fitness of cheats; leading to a lower proportion of cheats being maintained in the population (Fig. 4A).

Pyoverdine production is energetically costly and its ubiquity suggests isolates are iron limited in their natural environment (Dumas et al. 2013). We did not specifically measure iron levels in soil samples, as our intent here was to test whether social interactions can potentially explain variation in pyoverdine production, not to assess isolates responses to environmental iron availability. Pyoverdine production can be lost if alternative iron sources are available, but these nonproducers have retained functional pyoverdine receptors which are costly even when expressed at low levels (Marvig et al. 2014; Nguyen et al. 2014; Andersen et al. 2015). *Pseudomonas fluorescens* isolates are also known

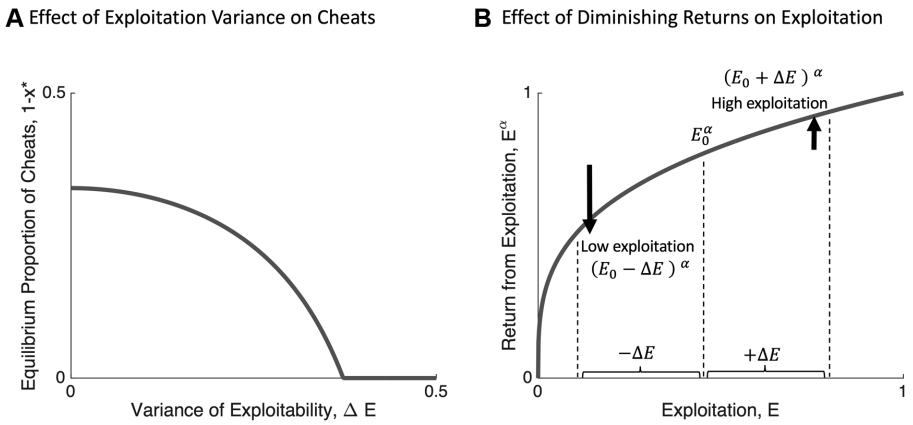


Figure 4. **(A) We plot the equilibrium proportion of cheat cells ($1 - x^*$) in the population as a function of variance of exploitability (ΔE), where high variance of exploitability means that cheater cells can exploit the extracellular factor released by cooperator cells in some local environments much better than in others. Here, we assume that the cooperators invest a fraction 0.5 of their private resources toward production of the extracellular factor, that the nonlinear return from the extracellular factor is diminishing (with shape term, $\alpha = 0.5$) and that mean exploitation is 0.5. (B) We show the relative return to cheater cells from exploitation in different local environments. If there is no cheat exploitation variance, then cheats receive a relative return of E_0^{α}, where E_0 is the expected exploitation and α quantifies the nonlinear return from the extracellular factor, which is assumed to be diminishing ($\alpha = 0.3$, here). If there is a nonnegligible variance in cheat exploitation, then we quantify this (symmetric) variance with the term ΔE. Cheats then experience two local environments with equal probability: a low exploitation environment where the relative return is $(E_0 - \Delta E)^{\alpha}$ and a high exploitation environment with relative return $(E_0 + \Delta E)^{\alpha}$. Due to the nonlinear fitness return, the low exploitation environment leads to a larger absolute change in fitness than the high exploitation environment (compare size of the arrows), and so an increased variance leads to a drop in the relative fitness of cheats.**

to carry multiple different receptors enabling the use of different structural forms of pyoverdine (Moon et al. 2008; Hartney et al. 2011; Ye et al. 2014). These observations suggest that nonproducers have lost production not because the trait is redundant in natural settings, but because they can extract sufficient iron from the pyoverdine of local producers. Nonproducing cheats can invade and persist in phylogenetically diverse, and spatially structured laboratory microcosms, and siderophore nonproducers have been identified in *Pseudomonas aeruginosa* populations in the CF lung and in marine populations of *Vibrio Spp.* (Joussett et al. 2013; Andersen et al. 2015; Lujan et al. 2015; Cordero et al. 2012). We do not specifically test the ability of nonproducers to invade populations of pyoverdine producers under laboratory conditions, as (1) these conditions are far removed from those in natural soil environments and (2) extrapolating the results of laboratory fitness assays to "real world" scenarios is problematic: demonstrating invasion of nonproducers in the laboratory would imply this happening in soil, even if it was not and vice versa. However, we do provide evidence that potential cheats can arise and persist in natural soil populations of bacteria, suggesting they may be a pervasive feature of natural bacterial populations. Cooperator-cheat dynamics are unlikely to be confined to production of siderophores, as bacteria and other microbes perform a range of potentially exploitable social traits (West et al. 2006). Cheating may not even be restricted to nonproducers: pyoverdine production is a continuous trait and we find co-occurrence of low- and high-level producers, allowing the possibility that low producers can exploit higher level producers (Jiricny et al. 2010; Ghoul et al. 2014b). Cheating behaviors have also been observed in a number of other natural systems including fruiting body formation in social amoeba, and rhizobia plant, plant pollinator, and cleaner fish mutualisms (Strassmann et al. 2000; Bronstein et al. 2004; Sachs et al. 2010; Bshary & Gutter. 2002).

While nonproducers can still extract iron from pyoverdine, we find that they can exploit on average only 52% of pyoverdine producers in the population. This variability contrasts sharply with most previous studies, where cheats are competed against isogenic cooperators they can exploit freely (Greig & Travisano 2004; Griffin et al. 2004; Diggle et al. 2007; Sandoz et al. 2007; Gore et al. 2009). In natural populations, social interactions occur between phylogenetically diverse isolates that may differ considerably in the trait of interest, and many others (Stefanic et al 2012; Stefanic et al. 2015; Kraemer et al. 2016). In our populations, we find that nonproducers are more likely to exploit closely related isolates than they are genetically dissimilar isolates (Fig. 3A). This suggests that potential cheats will be at an initial advantage when they arise in the population, as they will be surrounded by exploitable close relatives, but may fare less well as they encounter genetically different strains. Our results suggest that, if the ability to exploit is contingent on interacting with genetically

similar isolates, in genetically diverse natural populations, variation in the extent to which cheats can exploit cooperators is likely the norm.

We examined theoretically the consequences of this variation in exploitability for cheat-cooperator dynamics. We found that variation in exploitability reduced the mean fitness of cheats, and so led to a lower proportion of cheats being maintained in the population (Fig. 4A). In some cases, these can even lead to cheats being excluded from the population. The reason for this influence of exploitability stems from increased levels of cooperation leading to diminishing (nonlinear) benefits (Fig. 4B). Variance in exploitability leads to some cheats gaining increased benefits, and some cheats gaining decreased benefits. With traits such as siderophores, the benefits from increased cooperation are diminishing (Ross-Gillespie et al. 2007). This nonlinearity means that the absolute gain in fitness from high exploitation is lower than the absolute drop in fitness from low exploitation, a manifestation of Jensen's inequality for concave functions (Fig. 4B). Consequently, an increase in the variability of exploitation leads to a decrease in the mean fitness of cheats.

What factors underlie this variation in the extent to which nonproducers exploit producers? Natural *P. fluorescens* isolates are likely to produce different forms of pyoverdine, potentially preventing nonproducers from exploiting some producers. Pyoverdine is a structurally diverse molecule, with over 50 types identified in *Pseudomonads*, and is considered a "lock-key" or "gift-password" system involving receptor-molecule binding specificity (Meyer et al. 2007; Strassmann et al. 2011). This specificity allows for uninhibited uptake of pyoverdine provided isolates carry the appropriate receptor, and limited or no uptake if they do not (Meyer 2000; Meyer et al. 2002). This may explain why exploitation occurs more often between closely related isolates: they are more likely to harbor complementary receptors. The pyoverdine locus is under selection for diversification in *Pseudomonas aeurginosa*, with theory suggesting this may be a response to exploitation: cheats drive cooperators to produce new, exclusive structural forms of pyoverdine (Smith et al. 2005; Eldar 2011; Lee et al. 2012). This specificity allows cooperation to be directed preferentially toward close relatives, preventing cheats from exploiting the cooperative behavior (Inglis et al. 2016a).

We have focused on variation in the cooperative trait and its consequences for the extent to which potential cheats can exploit cooperators. However, we find that some cooperators produce bacteriocins that kill potential cheats. This suggests that other bacterial traits may influence the extent to which exploitation of cooperative behaviors can occur (Lyons et al. 2016). Most bacteria produce toxins that kill or inhibit the growth of closely related strains or species, and in *P. aeruginosa*, some of these toxins even target siderophore receptors (Baysse et al. 1999; Ghequire & De Mot 2014; Inglis et al. 2016b). There is little evidence that bacteriocin production has evolved as a mechanism to punish noncooperators but susceptibility of cheats to cooperators toxins will likely prevent successful exploitation. While this may occur only in a small percentage of interactions, bacteriocins are only one of an arsenal of competitive mechanisms employed by bacteria to exclude nonrelatives (Ruhe et al. 2013; Unterweger et al. 2014). Our results suggest that competitive mechanisms will also contribute to variability in the extent to which cheats can exploit cooperators in natural populations.

AUTHOR CONTRIBUTIONS

JBB, SAW, ASG and HC designed the study, JBB carried out data collection, JBB, SAW and ASG analyzed the data, GAC and SAW conceived the model, JBB, GAC, SAW and ASG wrote the paper.

ACKNOWLEDGMENTS

We would like to thank M. Ghoul, L. Santorelli, and S. Andersen for advice, and the NERC, the EPSRC, the ERC and the Royal Society for funding. The authors declare no conflict of interest.

DATA ARCHIVING

The doi for our data is https://doi.org/10.5061/dryad.36g6r

LITERATURE CITED

Andersen, S. B., R. L. Marvig, S. Molin, H. Krogh Johansen, and A. S. Griffin. 2015. Long-term social dynamics drive loss of function in pathogenic bacteria. Proc. Natl. Acad. Sci. 112:10756–10761.

Baysse, C., J. M. Meyer, P. Plesiat, V. Geoffroy, Y. Michel-Briand, and P. Cornelis. 1999. Uptake of pyocin S3 occurs through the outer membrane ferripyoverdine type II receptor of *Pseudomonas aeruginosa*. J. Bacteriol. 181:3849–3851.

Bourke, A. F. G. 2011. Principles of social evolution. In P. H. Harvey, R. M. May, C. H. Godfray, and J. A. Dunne, eds. Oxford series in ecology and evolution. Oxford Univ. Press, Oxford, U.K.

Bozdag, G. O., and D. Greig. 2014. The genetics of a putative social trait in natural populations of yeast. Mol. Ecol. 23:5061–5071.

Bronstein, J. L., R. Alarcon, and M. Geber. 2004. The evolution of plant-insect mutualisms. New Phytol. 172:412–428.

Bruce, J. B., S. A. West, and A. S. Griffin. 2017. Bacteriocin production mediates competition over resources and influences assembly of natural *Pseudomonas fluorescens* populations. J. Evol. Biol. 30(2):352–360.

Bshary, R., and A. S. Grutter. 2002. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. Anim. Behav. 63:547–555.

Colombo, C., G. Palumbo, J. He, R. Pinton, and S. Cesco. 2014. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. J Soils Sediments. 14:538–548.

Cordero, O. X., L. A. Ventouras, E. F. DeLong, and M. F. Polz. 2012. Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations. Proc. Natl. Acad. Sci. 109:20059–20064.

Cornforth, D. M., D. J. Sumpter, S. P. Brown, and A. Brannstrom. 2012. Synergy and group size in microbial cooperation. Am. Nat. 180:296–305.

Diggle, S. P., A. S. Griffin, G. S. Campbell, and S. A. West. 2007. Cooperation and conflict in quorum-sensing bacterial populations. Nature 450:411–414.

Dumas, Z., A. Ross-Gillespie, and R. Kummerli. 2013. Switching between apparently redundant iron-uptake mechanisms benefits bacteria in changeable environments. Proc. Biol. Soc. 280:20131055–20131055.

Eldar, A. 2011. Social conflict drives the evolutionary divergence of quorum sensing. Proc. Natl. Acad. Sci. 108: 13635–13640.

Frank, S. A. 2010. Microbial secretor-cheater dynamics. Phil. Trans. R. Soc. B. 365:2515–2522.

Ghequire, M. G. K., and R. De Mot. 2014. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. FEMS Microbiol. Rev. 38:523–568.

Ghoul, M., A. S. Griffin, and S. A. West. 2014a. Toward an evolutionary definition of cheating. Evolution 68:318–331.

Ghoul, M., S. A. West, S. P. Diggle, and A. S. Griffin. 2014b. An experimental test of whether cheating is context dependent. J. Evol. Biol. 27: 551–556.

Ghysels, B., B. T. M. Dieu, S. A. Beatson, J. P. Pirnay, U. A. Ochsner, M. L. Vasil, and P. Cornelis. 2004. FpvB, an alternative type I ferripyoverdine receptor of Pseudomonas aeruginosa. Microbiology 150:1671–1680.

Gore, J., H. Youk, and A. van Oudenaarden. 2009. Snowdrift game dynamics and facultative cheating in yeast. Nature 459:253–256.

Greenwald, J., F. Hoegy, M. Nader, L. Journey, G. L. A. Mislin, P. L. Graumann, and I. J. Schalk. 2007. Real time fluorescent resonance energy transfer visualization of ferric pyoverdine uptake in *Pseudomonas aeruginosa*. J. Biol. Chem. 282:2987–2995.

Greig, D., and M. Travisano. 2004. The prisoner's dilemma and polymorphism in yeast SUC genes. Proc. Biol. Sci. 271(Suppl_3):S25–S26.

Griffin, A. S., and S. A. West. 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 302:634–636.

Griffin, A. S., S. A. West, and A. Bucking. 2004. Cooperation and competition in pathogenic bacteria. Nature 430: 1024–1027.

Harrison, F. 2013. Bacterial cooperation in the wild and in the clinic: are pathogen social behaviors relevant outside the laboratory? Bioessays 35:108–112.

Hartney, S. L., S. Mazurier, T. A. Kidarsa, M. C. Quecine, P. Lemanceau, and J. E. Loper. 2011. TonB-dependent outer-membrane proteins and siderophore utilization in Pseudomonas fluorescens Pf-5. Biometals 24:193–213.

Inglis, R. F., S. P. Brown, and A. Buckling. 2009. Spite versus cheats: competition among social strategies shapes virulence in *Pseudomonas aeruginosa*. Evolution 66:3472–3484.

Inglis, R. F., J. M. Biernaskie, A. Gardner, and R. Kummerli. 2016a. Presence of a loner strain maintains cooperation and diversity in well-mixed bacterial communities. Proc. Biol. Sci. 283:20152682.

Inglis, R. F., P. Scanlan, and A. Buckling. 2016b. Iron availability shapes the evolution of bacteriocin resistance in *Pseudomonas aeruginosa*. ISME J. 10:2060–2066.

Jiricny, N., S. P. Diggle, S. A. West, B. A. Evans, G. Ballantyne, A. Ross-Gillespie, and A. S. Griffin. 2010. Fitness correlates with the extent of cheating in a bacterium. J. Evol. Biol. 23:738–747.

Jones, E. I., M. E. Afkhami, E. Akcay, J. L. Bronstein, R. Bshary, M. E. Frederickson, K. D. Heath, J. D. Hoeksema, J. H. Ness, M. S. Pankey, et al. 2015. Cheaters must prosper: reconciling theoretical and empirical perspectives on cheating in mutualism. Ecol. Lett. 18:1270–1284.

Jousset, A., N. Eisenhauer, E. Materne, and S. Scheu. 2013. Evolutionary history predicts the stability of cooperation in microbial communities. Nat. Commun. 4:2573.

Kiers, E. T., R. A. Rousseau, S. A. West, and R. F. Denison. 2003. Host sanctions and the legume-rhizobium mutualism. Nature 425:78-81.

Koschwanez, J. H., K. R. Foster, and A. W. Murray. 2011. Sucrose utilization in budding yeast as a model for the origin of undifferentiated multicellularity. PLoS Biol. 9: e1001122.

Kraemer, S. A., S. Wielgoss, F. Fiegna, and G. J. Velicer. 2016. The biogeography of kin discrimination across microbial neighbourhoods. Mol. Ecol. 25:4875–4888.

Kümmerli, R., and A. Ross Gillespie. 2013. Explaining the sociobiology of pyoverdin producing *Pseudomonas*: a comment on Zhang and Rainey (2013). Evolution 68:3337–3343.

Lee, W., M. van Baalen, and V. A. Jansen. 2012. An evolutionary mechanism for diversity in siderophore producing bacteria. Ecol. Lett. 15:119–125.

Lindsay, W. L., and A. P. Schwab. 1982. The chemistry of iron in soils and its availability to plants, J Plant Nutr, 5:4–7, 821–840.

Lujan, A. M., P. Gomez, and A. Buckling. 2015. Siderophore cooperation of the bacterium *Pseudomonas fluorescens* in soil. Biol. Lett. 11:20140934–20140934.

Lyons, N. A., B. Kraigher, P. Stefanic, I. Mandic-Mulec, and R. Kolter. 2016. A combinatorial kin discrimination system in *Bacillus subtilis*. Curr. Biol. 26:733–742.

Marvig, R. L., S. Damkiaer, S. M. H. Khademi, T. M. Markussen, S. Molin, and L. Jelsbak. 2014. Within-host evolution of *Pseudomonas aeruginosa* reveals adaptation toward iron acquisition from hemoglobin. mBio 5:e00966–14.

Meyer, J. M. 2000. Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* species. Arch. Microbiol. 174:135–142.

Meyer, J. M., V. A. Geoffroy, N. Baida, L. Gardan, D. Izard, P. Lemanceau, W. Achouak, and N. J. Palleroni. 2002. Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. Appl. Environ. Microbiol. 68:2745–2753.

Meyer, J.-M., C. Gruffaz, V. Raharinosy, I. Bezverbnaya, M. Schäfer, and H. Budzikiewicz. 2007. Siderotyping of fluorescent *Pseudomonas*: molecular mass determination by mass spectrometry as a powerful pyoverdine siderotyping method. Biometals. 21:259–271.

Moon, C.D., X. Zhang, S. Matthijs, M. Schäfer, H. Budzikiewicz, and P. B. Rainey. 2008. Genomic, genetic and structural analysis of pyoverdine-mediated iron acquisition in the plant growth-promoting bacterium Pseudomonas fluorescens SBW25. BMC Microbiol. 8:7.

Nguyen, A. T., M. J. O'Neill, A. M. Watts, C. L. Robson, I. L. Lamont, A. Wilks, and A. G. Oglesby-Sherrouse. 2014. Adaptation of iron homeostasis pathways by a *Pseudomonas aeruginosa* pyoverdine mutant in the cystic fibrosis lung. J. Bacteriol. 196:2265–2276.

Pepper, J. W. 2000. Relatedness in trait group models of social evolution. J. Theor. Biol. 206:355–368.

Riehl, C., and M. E. Frederickson. 2016. Cheating and punishment in cooperative animal societies. Phil. Trans. R. Soc. B. 371:20150090.

Riley, M. A., and D. M. Gordon. 1999. The ecological role of bacteriocins in bacterial competition. Trends Microbiol. 7:129–133.

Ross Gillespie, A., A. Gardner, S. A. West, and A. S. Griffin. 2007. Frequency Dependence and Cooperation: Theory and a Test with Bacteria. Am. Nat. 170(3):331–342.

Ruhe, Z. C., D. A. Low, and C. S. Hayes. 2013. Bacterial contact-dependent growth inhibition. Trends Microbiol. 21:230–237.

Sachs, J. L., M. O. Ehinger, and E. L. Simms. 2010. Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. J. Evol. Biol. 23:1075–1089.

Sandoz, K. M., S. M. Mitzimberg, and M. Schuster. 2007. Social cheating in *Pseudomonas aeruginosa* quorum sensing. Proc. Natl. Acad. Sci. 104:15876–15881.

Sharp, S. P., A. McGowan, M. J. Wood, and B. J. Hatchwell. 2005. Learned kin recognition cues in a social bird. Nature 434:1127–1130.

Smith, E. E., E. H. Sims, D. H. Spencer, R. Kaul, and M. V. Olson. 2005. Evidence for diversifying selection at the pyoverdine locus of *Pseudomonas aeruginosa*. J. Bacteriol. 187:2138–2147.

Stefanic, P., F. Decorosi, C. Viti, J. Petito, F. M. Cohan, and I. Mandic-Mulec. 2012. The quorum sensing diversity within and between ecotypes of *Bacillus subtilis*. Environ. Microbiol. 14:1378–1389.

Stefanic, P., B. Kraigher, N. A. Lyons, R. Kolter, and I. Mandic-Mulec. 2015. Kin discrimination between sympatric *Bacillus subtilis* isolates. Proc. Natl. Acad. Sci. 112:14042–14047.

Strassmann, J. E., Y. Zhu, and D. C. Queller. 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. Nature 408:965–967.

Strassmann, J. E., O. M. Gilbert, and D. C. Queller. 2011. Kin discrimination and cooperation in microbes. Ann. Rev. Microbiol. 65:349–367.

Strassmann, J. E., and D. C. Queller. 2014. Privatization and property in biology. Anim. Behav. 92:305–311.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725–2729.

Unterweger, D., S. T. Miyata, V. Bachmann, T. M. Brooks, T. Mullins, B. Kostiuk, D. Provenzano, and S. Pukatzki. 2014. The Vibrio cholerae type VI secretion system employs diverse effector modules for intraspecific competition. Nat. Commun. 5:3549.

Varma, A., and S. B. Chincholkar, eds. 2007. Soil biology, Volume 12: Microbial siderophores. Springer-Verlag, Berlin.

Wandersman, C., and P. Delepelaire. 2004. Bacterial iron sources: from siderophores to hemophores. Ann. Rev. Microbiol. 58:611–647.

Wang, M., A. L. Schaefer, A. A. Dandekar, and E. P. Greenberg. 2015. Quorum sensing and policing of *Pseudomonas aeruginosa* social cheaters. Proc. Natl. Acad. Sci. 112:2187–2191.

West, S. A., and A. Bucking. 2003. Cooperation, virulence and siderophore production in bacterial parasites. Proc. Biol. Soc. 270:37–44.

West, S. A., A. S. Griffin, A. Gardner, and S. P. Diggle. 2006. Social evolution theory for microorganisms. Nat. Rev. Microbiol. 4:597–607.

West, S. A., S. P. Diggle, A. Buckling, A. Gardner, and A. S. Griffin. 2007. The social lives of microbes. Annu. Rev. Ecol. Evol. Syst. 38:53–77.

Ye, L., S. Matthijs, J. Bodilis, F. Hildebrand, J. Raes, and P. Cornelis. 2014. Analysis of the draft genome of Pseudomonas fluorescens ATCC17400 indicates a capacity to take up iron from a wide range of sources, including different exogenous pyoverdines. Biometals 27:633–644.

Associate Editor: B. Koskella
Handling Editor: M. Noor

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Growth of non-producer isolates in iron-limited CAA media and in iron-limited CAA media supplemented with purified pyoverdine (Sigma).
Figure S2. Histograms of levels of pyoverdine in producer supernatant for cross-feeding experiments resulting in a significant growth increase versus no significant growth increase for non-producers. N=1656 cross-feeding assays.
Table S1. PCR and Sequencing Primers and Annealing temperatures.
Table S2. Tukey multiple comparisons of means.
Table S3. Comparison of non-producer growth in iron-limited media and iron limited media supplemented with purified pyoverdine.