Spatial and social structure of rewilded laboratory mice

Caleb C. Vogt[1*], Matthew N. Zipple[1], Daniel D. Sprockett[2], Caitlin H. Miller[1], Summer H. Hardy[1], Matthew Arthur[1], Adam M. Greenstein[1], Melanie Colvin[1], Andrew H. Moeller[2], Michael J. Sheehan[1*]

[1]Laboratory for Animal Social Evolution and Recognition, Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

[2]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853

*Correspondence to: ccv26@cornell.edu, msheehan@cornell.edu

HIGHLIGHTS

- We describe emergent spatial and social structures of rewilded C57BL/6J (C57) lab mice across replicated trials in outdoor field enclosures and compare them to wild-derived outbred mice
- Both C57 and outbred males rapidly establish and maintain territories
- C57 females explore the field enclosures substantially more than any other group
- With the exception of C57 females, most mice spent the majority of their recorded time alone
- The resulting societies formed by C57 mice are less modular, more densely connected, and less stable than those formed by wild-derived outbred mice

Keywords: Rewilding, laboratory mice, space use, social structure, territoriality

Abstract

As an essential biomedical model organism, house mice have been studied intensely under laboratory conditions, yet they evolved to survive and reproduce in complex and dynamic environments. There has been recent interest in the study of 'rewilded' mice reared in complex outdoor environments, particularly for understanding the brain and behavior. Yet little work has examined lab mouse behavior under free-living conditions. Here, we characterize the emergent spatial and social structure of replicated populations of C57BL/6J (C57) mice over 10 days in large outdoor field enclosures and compare them to populations of recently wild-derived outbred house mice under the same conditions. We observed shared aspects of space use and social structure across all trials but found that C57 societies differed from those emerging from outbred mice across multiple dimensions. Males of both genotypes rapidly established and then defended territories. Female C57 mice spent more time with other individuals and explored more space relative to all other groups. These behavioral differences resulted in C57 mice rapidly forming less stable, but more densely connected, social networks than outbred wild-derived mice. These data suggest that laboratory domestication has had larger effects on female mouse social organization than their male counterparts. Importantly, this work demonstrates that C57 mice recapitulate many, but not all, aspects of social structures generated by wild mice in outdoor conditions. Rewilding allows for tractable, replicable, and ecologically realistic approaches to

studying mouse behavior and can facilitate the study of the biological basis of higher order social organization.

INTRODUCTION

Laboratory house mice are the premier model organism in biomedical research due to their small size, rapid breeding cycle, and the ready deployment of precise experimental manipulations using powerful genetic and neurobiological tools[1–4]. Studying mice in the lab affords tremendous experimental control allowing for the fine-scale dissection of proximate mechanisms across a range of biological fields including genetics, physiology, and neuroscience[2,5,6]. While controlled conditions are necessary for many experiments, there has been a growing recognition that indoor lab environments limit our ability to understand many complex biological processes[7–9]. This motivation is especially strong in neuroscience, where a growing number of researchers have highlighted a need to study the brain and behavior in enriched environments that can elicit an animal's full repertoire of natural behaviors[10–16]. Constrained lab environments inherently limit the study of patterns of space use or social behavior that require realistic natural spatial scales relevant to the organism. Even relatively large and enriched lab settings[17–19] fail to capture many of the relevant features of social interactions and social structures inferred by studies of wild mouse populations to be important to mouse natural history, such as territoriality and space use[20–24].

An immediate solution is to study the behavior of lab mice in large natural spaces. There is a long history of studies utilizing large enclosures to study the population biology of mice under free-living conditions[25–35]. These studies tend to use feral or wild-derived populations of outbred house mice and find that male mice establish and aggressively defend territories occupied by several females and their offspring. Fully adult males are most often associated with high quality territories, while juveniles and subadults typically aggregate in lower quality spaces within the environment[26,36,37]. Adult females also aggressively defend territories against male and female intruders[38–41]. However, multiple lines of evidence demonstrate that lab mouse strains commonly used for behavioral research differ from their wild counterparts in aspects of their behavior and physiology due to generations of inbreeding, artificial selection for fecundity and docility, and rearing in chronically impoverished cage environments[2,42–46]. It is not known if lab mice adopt similar social structures to wild mice under natural conditions. Though a small number of studies have studied rewilded lab mice in outdoor enclosures[47–51], they have not detailed the social behavior or emergent social structure of these animals. As a result, fundamental features of lab mouse behavior under free-living natural conditions remains poorly understood.

Characterizing the behavior of individuals and emergent social structures of lab mice under free-living conditions are critical first steps for 'rewilding' the field of neuroscience. The consistency of social structures under similar conditions has been poorly explored in mice and other animals, yet common garden studies of social organization have the potential to reveal which factors shape animal societies. Realized social organizations in populations may be highly variable if they are determined by

idiosyncratic individual behaviors and historical contingencies. Alternatively, populations with similar initial ecological and demographic conditions may reliably generate similar social structures, suggesting that the biological basis of social organization is amenable to study.

Here we report the space use and social behavior of replicated populations of the common laboratory strain C57BL/6J (C57) in large outdoor field enclosures located in upstate New York, USA. We also conducted identical, simultaneous trials using outbred wild-derived house mice. Thus, our dataset both describes how lab mice freely behave in large outdoor spaces and allows for a direct comparison of the similarities and differences in behavior between C57 and genetically outbred wild-derived mice as well as their emergent social structures under the same conditions.

RESULTS

Rewilded mouse behavior and social structure in the field

Field studies of wild populations provide a powerful means to link aspects of organismal biology to selection, but are typically hampered by a lack of replication[52,53]. Enclosure studies conducted over short, but biologically relevant, periods provide an opportunity to observe replicate populations across multiple trials. Over a three-month period (June 2020 - August 2020), we performed replicate trials to examine the emergent social organization generated in enclosures stocked with 10 female and 10 male house mice (*Mus musculus domesticus*) from the domesticated lab mouse strain C57 (n = 4) and outbred wild-derived house mice (n = 3). The mouse population density in our enclosure was ~0.034 mice per square meter, which falls within the range of typical population densities reported for wild mice[54]. Our outdoor field enclosures are approximately 9,000 times larger than the area of a standard laboratory mouse cage (**Fig. 1a**; **Fig. S1a-b**). Each field enclosure contained eight weather protected resource zones (made from 32-gallon rubber storage totes), which were equally distributed in a 2x4 grid. We supplied all resource zones with food and water accessible by the mice *ad libitum.* Additionally, we monitored the zones continuously over the trial period via an infrared video camera and a radio frequency identification (RFID) antenna placed beneath the sole entrance into the zone (**Fig. 1a**; **Fig S1c**). To initiate each trial, we placed mice into one of the eight resource zones with their same-sex cage mates in the evening shortly before sunset, meaning that all individuals started the trials in a resource zone in a social context.

Over the course of 10 days, mice explored the enclosures and resource zones, formed territories, and engaged in a variety of social interactions with conspecifics including courtship, mating, co-nesting, and fighting (**Fig. 1a**; **Video S1**). As the goal here is to identify the patterns of space use and social structure, we focus our analyses on the RFID dataset. We obtained high density sampling of mouse RFID reads for all trials (1,198,377 ± 102,782 RFID reads per trial; mean ± SEM) and a mean of 6,205 ± 236 RFID reads per mouse per day (**Table S1**). Mice were able to quickly traverse the distance between the zones despite the ground vegetation (minimum inter-zone travel time = 10 seconds, mean = 85.6 minutes, maximum = 16.4 hours; **Fig. S1d**). To convert instantaneous mouse RFID reads into estimates of how long mice spent in or around the zones, we grouped RFID reads into state events

with durations (**Fig. S1e**; see Methods for grouping procedure). The total number of visits to a zone strongly predicted the total estimated duration of time spent in a zone (Spearman's correlation, $R > 0.84$, $P < 0.001$ for all genotype and sex combinations; **Fig. S1f**). Using this approach, we estimated individual mouse location for a total of 5833.3 mouse hours across all trials (mean = 833.3 ± 52.1 hours per trial; **Table S1**). On average, we inferred that individual mice spent 4.28 ± 0.1 hours per day in the resource zones though we inferred a wide range of zone occupancy times from 12.2 seconds to 19.2 hours in a given day across all mouse days.

122

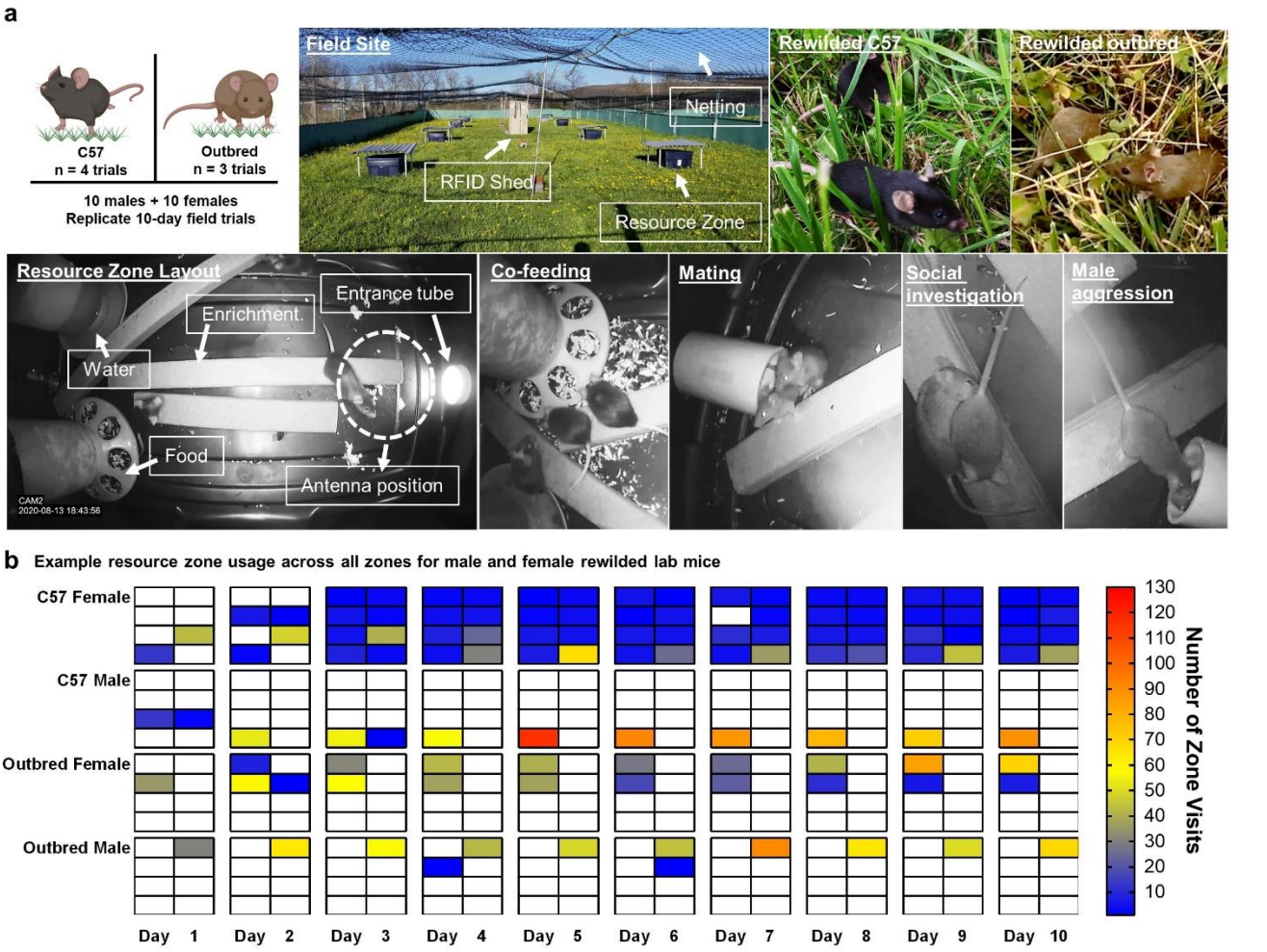

Figure 1: Field site and study design. (a) Experimental design for replicate populations of C57 and outbred mice in field enclosures. Photos demonstrate the layout of the field enclosures and the eight resource zones arranged in a 2x4 grid pattern. Resource zones had a single entrance tube and food and water towers provisioned *ad libitum.* A variety of behaviors were observed in the resource zones including co-feeding between females, mating and courtship, social investigation, and male-directed aggression towards intruders. Mouse schematics were created with BioRender. **(b)** Schematic of the resource zone locations (colored boxes) within the field enclosures (2x4 grids) showing typical patterns of zone visitation for four typical animals (rows) representing each sex and genotype across 10 days of activity (columns). White boxes show resource zones that mouse did not visit on that day of the trial.

Spatial structure of rewilded C57 and outbred mice

We first examined how mice utilized the space within the enclosures over the course of the 10-day trials. C57 females showed strikingly different space and movement patterns across several measures, as compared to C57 males, outbred males, and outbred females (**Fig. 1b**).

We estimated the minimum distance traveled per day for each mouse based on the distance and number of transitions made between distinct resource zones. Across sexes and genotypes, mice increased their daily distance travelled within the enclosure as a trial progressed ($F_{1,139.69}$ = 54.25, P < 0.0001; **Fig. 2a**), but overall C57 females travelled much further than all the other groups over the course of the entire trial (P < 0.01 for all comparisons; **Fig. S2a**). C57 females resembled other groups for the first few days, but then dramatically increased and maintained their greater minimum estimated travel distance relative to other groups starting on the fourth day of the trials (P < 0.05 for daily LMM model contrast estimates for Day 4 – 10; **Fig. 2a**).

C57 female travel was not limited to a few resource zones, but instead was widespread across the enclosure space. Across sexes and genotypes, mice visited an average of 2.34 ± 0.05 resource zones per day over the course of the trial, though patterns of zone visits varied over time and among individuals. The number of unique resource zones visited per individual per day was significantly influenced by time in the trial ($F_{1,133.29}$ = 30.65, P < 0.001), but this increase was driven entirely by the behavior of C57 females (P = 0.31 for non-C57 females; **Fig. 2b**). Although all mice explored an equivalently low number of resource zones during the first several days in the enclosure, by the fourth day C57 females had significantly increased exploration of the available zones compared to all other groups (P < 0.05 for daily LMM contrast estimates for Day 4 – 10), which did not differ in their extent of space use.

In addition to visiting more unique zones on average per day, C57 females visited a greater proportion of all possible zones over the course of the trial (**Fig. 2c**). By the final day of the trial C57 females had visited 6.27 ± 0.43 of the available zones, which is more than C57 males (4.29 ± 0.42; $t_{125.11}$ = -5.43, P < 0.0001), outbred females (4.1 ± 0.49; $t_{7.56}$ = -3.33, P = 0.011), and outbred males (3.46 ± 0.49; $t_{125.11}$ = 2.40, P = 0.017). Substantially more C57 females (44%, 17/38) visited all 8 resource zones compared to C57 males (7.69%, 3/39), outbred females (3.44%, 1/29), and outbred males (3.57%, 1/28) (generalized LMM: P < 0.05 for all comparisons).

These differences in the number of zones visited each day and cumulatively across the trial were not the result of C57 females spending more time in resource zones (P > 0.15 for sex and genotype main effects; **Fig. S2b**). Rather, C57 females displayed reduced fidelity to their most visited resource zone compared to other groups. Most individuals tended to favor a single resource zone, but C57 females show a much weaker bias towards spending time in their most occupied zone relative to males and outbred females (P < 0.05 for all comparisons; **Fig. 2d**).

Given that mice tended to prefer one zone, we next examined how mice established and maintained priority access to resource zones. We calculated a daily resource zone Priority Access Score (PAS) per mouse based on the duration of time a mouse spent in a zone relative to all other same-sex

individuals and examined how this score changed over time (**Fig. S2c-d**). Briefly, mice gained 1 point for each resource zone they fully monopolized or a fraction of a point for partial monopoly. Mice that failed to monopolize any zone (< 50%) were give a -1 penalty (see Methods for full description). Thus, for the 10-day trials reported here, strongly positive scores (near +10) indicate an individual consistently excluded others of the same sex from a single resource zone while strongly negative scores (near -10) indicate an individual was consistently excluded from most spaces. Very high scores (>>10) indicate individuals monopolized more than 1 zone. Scores closer to zero indicate individuals that share spaces to some extent with others of the same sex. Overall, females vary widely in the extent to which they establish and maintain priority access over resource zones such that the distribution of female PAS values were unimodal and centered near zero for both genotypes by the final day of the trial (**Fig. 2e**). Males, in contrast, settled into a largely bimodal population of males with high and low PAS values, demonstrating the presence of territorial males and males who failed to establish a territory within the population (**Fig. 2f**). Thus, for both genotypes our trial design reliably generates territorial behavior consistent with studies of wild house mice at similar densities.

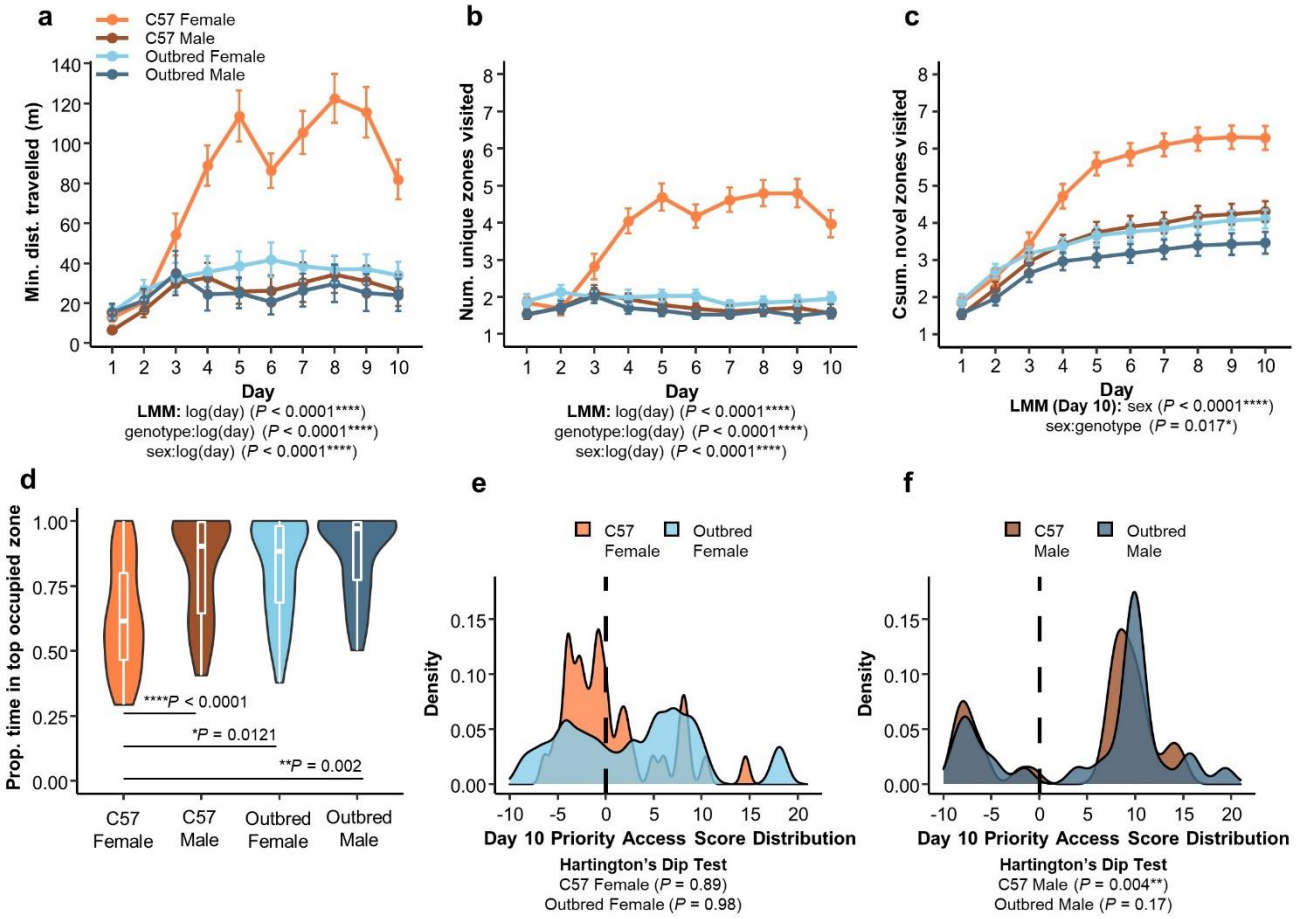

Figure 2: Spatial structure of rewilded C57 and outbred lab mice. C57 female mice differed from C57 males and outbred males and females on several metrics including **(a)** the estimated minimum distance travelled per day, **(b)** the number of resource zones visited per day, and **(c)** the cumulative number of novel zones visited over the entire trial period. **(d)** Proportion of the total time a mouse was observed across all zones spent in a mouse's top occupied zone (resource zones rank ordered by mouse occupancy time). **(e-f)** Distributions of cumulative Priority Access Scores after 10 days for female **(e)** and male **(f)** mice. Higher scores indicate the extent to which a mouse maintained majority access over one or more resource zones relative to same-sex conspecific competitors (see Methods).

177

Genotypes and sexes differ in the extent and nature of social interactions

We next examined how mice overlapped in space and time to determine to what extent individuals interact socially as well as the range of group compositions that arose. For each trial we estimated the time spent in each of the 120 possible combinations of the 10 males and 10 females in the experiment (**Fig. 3a**). We inferred individuals were simultaneously present in a resource zone whenever estimated visitation bout durations directly overlapped with other mice.

Most of the time that mice spent in resource zones was spent alone (range 56-87% solitary mouse time per trial; **Fig. 3a)**, but the proportion of time that individuals spent alone was strongly predicted by sex and genotype. On average, males spent a greater proportion of recorded time in resource zones alone than females ($F_{1,126}$ = 56.5, P < 0.0001; **Fig. 3b**). Indeed, we frequently observed males sitting in the resource zones oriented toward the entrance seemingly waiting for other mice to visit (**Video S1**). Outbred mice were more likely to be alone in the zones than C57 mice ($F_{1,5}$ = 48.51, P = 0.0009; **Fig. 3b**). Overall, outbred males were especially likely to spend time alone compared to other individuals; all of them (29/29) spent more than 50% of their total recorded time alone. In comparison 77% of outbred females (23/30), 75% of C57 males (30/40), and only 18% of C57 females (7/40) spent the majority of their recorded time alone. Given the interest in the biology of social isolation in mice[55–59], it is notable that when given the opportunity to freely interact, many mice opted instead to spend a significant portion of their time alone over the course of their trial.

Though individuals spend a large portion of their time in the resource zones by themselves, we estimated more than 1500 mouse hours of social interactions across the seven trials, defined as time with two or more mice in the zone. Dyadic interactions accounted for the majority of estimated social interaction time in both genotypes (75.3% in C57, 87.1% in outbred), though larger aggregations of mice were also detected in all trials (**Fig. 3a**). On average, females spent a greater portion of their recorded time in social groups than males, both in terms of mixed-sex ($F_{1,126.05}$ = 31.84, P < 0.0001; **Fig. S3a**) and same-sex groups ($F_{1,126.18}$ = 25.85, P < 0.0001; **Fig. S3b**). Compared to outbred mice, C57 mice were more likely to be engaged in both mixed-sex ($F_{1,4.99}$ = 33.27, P = 0.002; **Fig. S3a**) or same-sex groups ($F_{1,5.14}$ = 15.19, P = 0.011; **Fig. S3b**). Most mice (75.6%, n = 102/135) spent >50% of their recorded social time in mixed sex groups. The relative proportion of social time in same-sex versus mixed-sex groups did not differ between sexes or genotypes (P > 0.67; **Fig. 3c**).

Compared to females, males showed a notably wider range of relative time spent in mixed sex groups (**Fig. 3c)**. The proportion of social time in mixed versus same-sex social interactions among males is inversely correlated with their resource zone Priority Access Score rank within their trial ($F_{1, 56.77}$ = 46.72, P < 0.0001; **Fig. 3d**). That is, males that monopolize resource zones spend relatively more of their social time with females compared to males that failed to gain priority access to resource zones, consistent with hypothesized benefits of territoriality[60–62]. The slope of the relationship differed significantly between genotypes, with outbred males showing a steeper relationship between Priority Access Score rank and time spent with females ($F_{1, 56.8}$ = 7.23, P = 0.0094; **Fig. 3d**), suggesting that the benefits of territoriality are especially strong among outbred mice.

We next investigated how long individuals tended to interact with each other in social grouping events across the course of the trial. Most interactions tended to be relatively brief and became shorter in duration over the course of the trials (**Fig. 3e** and **Fig. S3c-d**). The length of mixed-sex interactions was shorter and decreased more strongly over time in outbred mice (genotype: $F_{1, 7.1}$ = 15.343, P = 0.006; genotype:time interaction: $F_{1, 19722}$ = 24.19, P < 0.0001; **Fig. S3c**). The length of same-sex interactions also decreased over time for both female-female ($F_{1,9152}$ = 124.26, P < 0.0001; **Fig. S3d**) and male-male ($F_{1, 1862}$ = 43.9, P < 0.0001; **Fig. 3e**) interactions. This decline was especially stark for males, who rarely interacted after territories were established during the first few days (**Fig. 3e**). Overall, male-male interactions were briefer in outbred compared to C57 males ($F_{1, 21.65}$ = 6.42, P = 0.019). Half of all time spent in male-male interactions by outbred males had elapsed within the first ~30 minutes of the trials, showing the remarkably quick deterioration of social relationships among cage mates once they were placed outside. The frequency of detected interactions also varied over the course of the trials, with male-male interactions becoming especially sparse after the first few days of the trials after territories had been established (**Fig. 3e**). The increasingly sparse and very brief interaction among males reflect the territoriality dynamics of males in these trials, which readily chase other males away from their monopolized zones (**Video S1**).

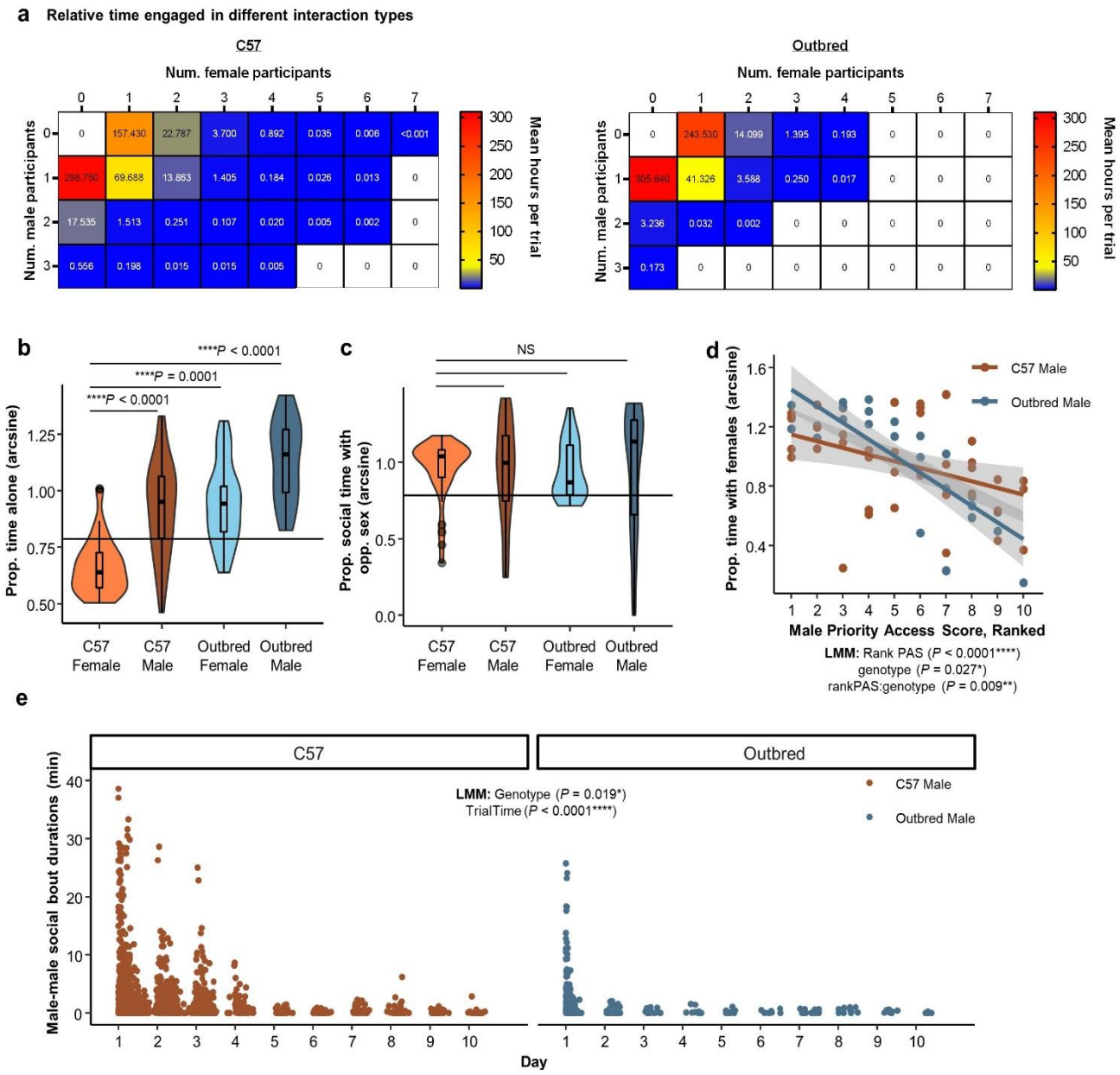

Figure 3: Social interactions and group compositions of rewilded mice. (a) Contour plot of average duration of trial time spent in different male and female group compositions. Only realized group compositions are shown across the two trials. **(b)** Proportion of observed time spent alone. The horizontal line represents the arcsine transformed 50% level. **(c)** Proportion of social time spent in groups with at least one member of the opposite sex. The horizontal line represents the arcsine transformed 50% level. **(d)** Relationship between the proportion of social time males spent in mixed sex groups and his ranked Priority Access Score on Day 10 (cumulative sum of all daily PAS values) for C57s (R = -0.41) and outbred (R = -0.81) males. **(e)** Male-male social grouping events were shorter and less frequent in outbred mice compared to C57 mice. For visualization purposes, the y-axis is cut off at 40 min (a small number of long interactions are inferred very early in the trial after mice are initially placed into resource bins, n = 1,865 events shown out of 1,872 total events).

Distinct social networks emerge between C57 and outbred mice

To investigate the emergent group structure of both genotypes, we analyzed the total and daily networks formed for each trial. Overall, C57 mice formed more connected networks than outbred mice, a difference which was largely driven by high levels of C57 female sociability (**Fig. 4a-b**). Outbred networks increased in the number of graph components – the portions of the network disconnected from each other – over time (genotype:log(day), $F_{1,61} = 17.02$, $P < 0.001$; **Fig. S4a**), reflecting the demic structure reported for many wild mouse populations[23,63,64]. Over time, the network edge density – a measure of the proportion of edges actually observed out of all possible edges in the network – increased in C57 social networks, but not in outbred networks (genotype:log(day), $F_{1,61}$, $P < 0.0001$; **Fig. S4b**).

Females of both genotypes had high degree centrality measures compared to their respective males, indicating females form key connections within mouse social networks. There was a significant three-way interaction between sex, genotype, and time, such that C57 females rapidly increased their network centrality measures compared to all other sex and genotype combinations (genotype:sex:log(day), $F_{1,133.83} = 6.66$, $P = 0.011$; **Fig. 4c**). Thus, many of the differences we see in social networks between the genotypes is driven by the propensity of C57 females to engage socially with many distinct individuals.

Social networks as a whole can be more or less centralized as a function of the individual node-level centrality measures, with more centralized networks having shorter distances on average between individuals. We examined the graph-level eigenvector centrality between C57 and outbred networks and found a significant interaction between genotype and time in the trial ($F_{1,61} = 17.02$, $P = 0.0001$; **Fig. S4c**). In other words, C57 networks gradually rose in their level of centralization over time while outbred networks stayed relatively constant. By the final day of the trial, C57 mice met many more of the available social partners present in the enclosures as compared to outbred mice (genotype: $F_{1,5} = 12.16$, $P = 0.017$; **Fig. 4d**), who failed, on average, to ever meet more than 50% of the potential social partners. Intriguingly, females of both genotypes showed high levels of vertex page rank scores, indicating that information flow through the network is more likely to move through females than males (sex:genotype:log(day): $F_{1,132.01} = 5.33$, $P = 0.023$; **Fig. 4e**).

Finally, we analyzed the extent to which social networks for each day of the trial predicted the social network structure on the final day of the trial. We found that outbred social networks were much more stable over time compared to C57 networks. For every outbred trial, the social network on the first day of the trial – and every day thereafter – was strongly predictive of the final realized social structure on Day 10 (MRQAP test, $P < 0.0001$, Bonferroni correction; **Fig. 4f**). In contrast, no C57 trial social network on Day 1 was strongly associated with the final network structure, and three out of four trials did not significantly predict the final social network until Day 5 of the trial.

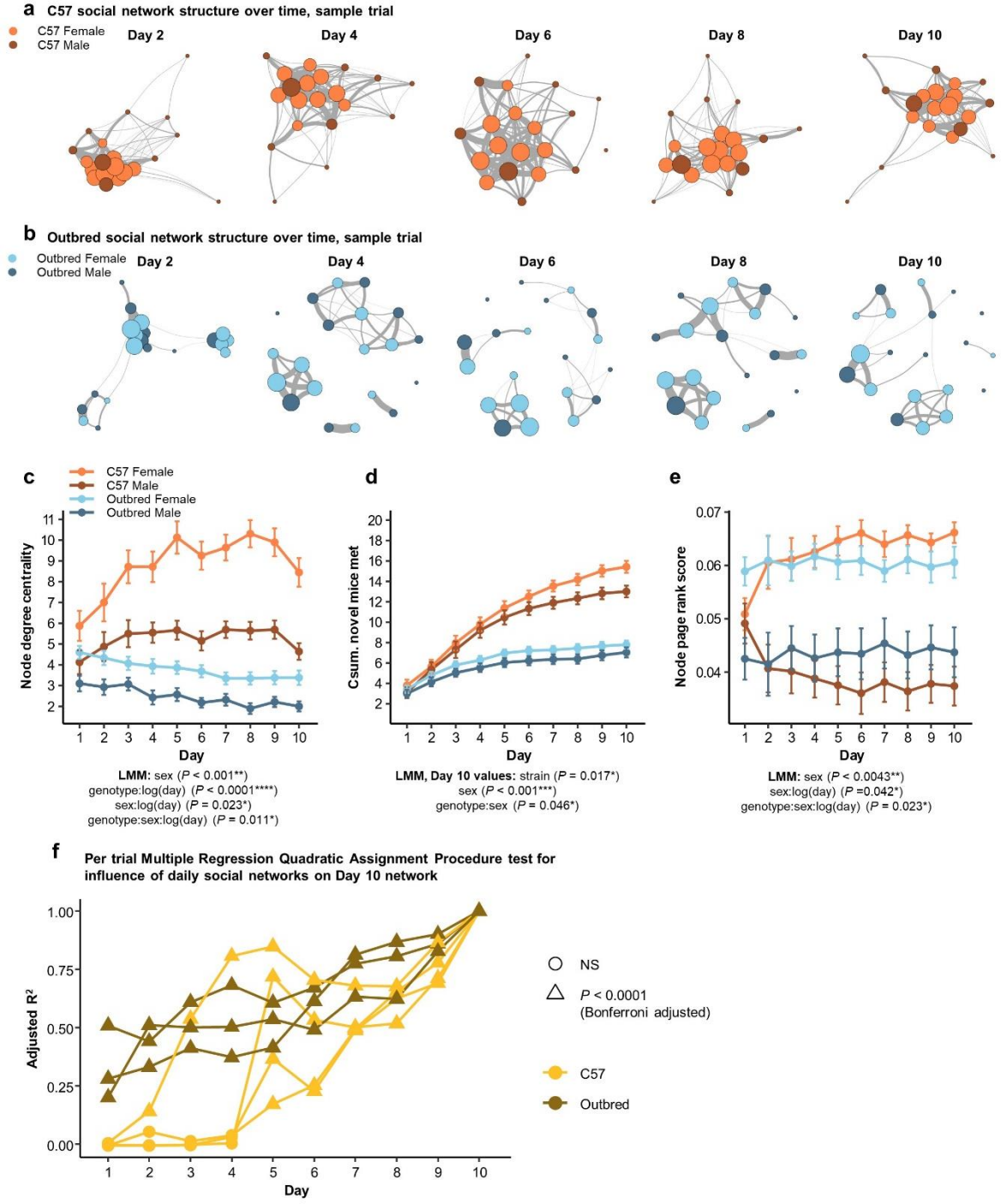

Figure 4: Social network structure of rewilded lab mice. (a-b) Daily social networks from an example C57 trial **(a)** demonstrates a typical pattern of persistently high female interconnectivity while an example outbred trial **(b)** demonstrates increasing network modularity over time. The size of connections between nodes represents the edge weight. Node sizes reflect the node edge strength, or the sum of all edge weights for a single node. **(c)** Node degree centrality over time show significant strain and sex interaction effects, with females of both strains having higher network centrality scores than males. **(d)** C57 mice met a majority of the available novel social partners by the final day of the trial, while outbred mice did not. **(e)** Both C57 and outbred females exhibited high page rank scores relative to males of either genotype, indicating that females serve as major conduits of information flow through the networks. **(f)** Outbred networks on each day of every trial are highly predictive of the final network structure on Day 10. C57 social networks are slower to stabilize.

DISCUSSION

Our replicated field experiments demonstrate that C57 lab mice broadly recapitulate the behaviors of wild-derived mice in free living conditions but have different emergent social structure largely due to females being more exploratory. The organization of mammal societies is influenced by ecological[65–67], demographic[68–71], and phylogenetic factors[72–74]. Our experiment controlled resource distribution and demographic composition of mice across trials. Thus, these data show that genotype can have a strong effect on social structures in mammals[75,76]. These data also highlight the flexibility of mouse social behaviors across diverse ecological and demographic conditions. For example, in contrast to lab studies at high density, which identify dominance hierarchies among males[18,19,77], the males in our lower density populations consistently formed and defended territories (**Fig. 2**). While our experiment only examined one set of ecological and demographic conditions, it demonstrates an approach in which variables including food resources, defensibility of spaces, and demographic compositions are all easily tunable.

What drives the difference that we saw in female space use across our trials (**Fig. 2**)? Space use in female mammals is often predicted by intra-sexual competition for food resources and nest sites[78], but resource availability and population density were identical across trials in our study. This suggests either that there is an innate difference in behavior between C57 and wild-derived females and/or that they respond differently to the social conditions present in our trials. In feral house mice, infanticide risk from both male and female conspecifics is thought to be a major driver of social behavior in females[79–81]. As a result, wild female house mice will aggressively defend space from other females[38–41,82] . C57 mice have been domesticated to live in cages at high densities, especially among females, and this is associated with lower female aggression compared to wild mouse genotypes[42]. Differences in relative tolerance of other females may be a key driver of the observed differences in social organization between C57 and outbred females in this study. An additional explanation for the behavior in C57 females may stem from the interactions of males and females in the trials. In the trials reported here, C57 females interact with C57 males while outbred females interacted with outbred males. Thus, male genotype could conceivably drive differences in patterns of female behavior. As one example, consider how genetic diversity among males in a trial may influence behavior. Whereas individuals in the outbred trials are genetically heterogenous and distinct, all the C57 mice are (essentially) genetically identical. Female mice respond to variation in perceived relatedness between themselves and males[33,83,84] and could potentially attend to how they perceive males to be related to each other. Understanding how innate behavioral differences among genotypes versus emergent properties generated by social interactions work together to shape mammalian societies is an exciting future direction that can be addressed with rewilded mouse studies.

Male space use in rodents and other mammals is frequently linked to patterns of female space use[78,81]. Yet despite differing patterns of female space use between genotypes, the male spatial and social structures were very similar, highlighting that some aspects of social organization are relatively less sensitive to other features of a population's socioecology. Perhaps one of the most striking

features of our study is the speed in which male-male social interactions deteriorate and decrease in frequency, especially among outbred males (**Fig. 3e**). Previous studies of wild mouse behavior have reported males will defend territories and attempt to monopolize spaces and exclude other males[25,29]. Though in low complexity environments or at high densities males may form dominance hierarchies[19,25]. The formation and consequences of dominance hierarchies among male mice have been the subject of recent study in the lab[19,77,85], though our results suggest that when given ample and defensible spaces male mice will tend to avoid interacting with others and form individual territories rather than a dominance hierarchy. The flexibility of house mouse social structure under different conditions has undoubtedly been important for their ecological success across diverse commensal and natural environments[20,23,86,87].

We identified not only consistent average differences in the behavior of individuals between genotypes but also differences in the higher-level social organizations of C57 lab mice and their wild-derived outbred counterparts (**Fig. 4**). Studies of social structures tend to come from idiosyncratic populations living in the wild, meaning that studies of social behavior in natural conditions are rarely replicated[52,88,89]. Studies of free-living populations are critically important, but this non-replicability makes understanding the specific genetic, neurobiological, ecological, and demographic factors influencing complex behavior challenging. The repeatability of social organization demonstrated here suggests that future work manipulating aspects of physiology or neural function in rewilded mice will offer a unique opportunity to study not just differences in individual behavior, but also how those behaviors reliably influence society.

Consent for Publication

All authors have read and approved this manuscript for publication.

Acknowledegments

We thank Melissa R. Warden for helpful discussions during the planning of the experiment. We thank Gary Olz and Russel L. Ligon for assistance in preparing the field site. Funding was provided by Cornell University (Neurobiology and Behavior Departmental Grant to CCV), the USDA (Hatch Grant, NYC-191428 to MJS), and the NIH (R35 GM138284 to AHM).

Author contributions

1) Conceptualization, 2) Study Design, 3) Methodology Design, 4) Field Work, 5) Data Curation & Code, 6) Data Analysis, 7) Figure Creation, 8) Writing – Original Draft, 9) Writing – Review & Editing, 10) Supervision, 11) Funding Acquisition. CCV: 1,2,3,4,5,6,7,8,9,10,11. MNZ: 6,9. DDS: 4,9. CHM: 4,9. SHH: 6. MA: 6. AMG: 6. MC: 4. AJM: 4,9,10,11. MJS: 1,2,4,6,7,8,9,10,11.

Data Availability

All data, statistical outputs, and R code for recreating figures and analyses are available on Zenodo (https://doi.org/10.5281/zenodo.6425497).

METHODS

Ethical statement

All procedures conformed to guidelines established by the U.S. National Institutes of Health and have been approved by the Cornell University Institutional Animal Care and Use committee (IACUC: Protocol #2015-0060).

Animals

We examined two genotypes of *M. m. domesticus,* C57BL/6J (C57) and wild-derived outbred mice. C57 mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). Outbred mice were derived from strains generated through distinct initial pairings of wild mice from Saratoga Springs, NY, USA, trapped by M.J.S in 2013. These mice are genetically related to The Jackson Laboratory wild-derived mouse strains SarA/NachJ (#035346), SarB/NachJ (#035347), and SarC/NachJ (#035348) mice which are descended from the same wild caught group of mice.

Study design and field site description

All field work was conducted at Cornell University's Liddell Laboratory Field Station in Dryden, New York, USA from May 2020 to August 2020. Male (n = 10 per trial) and female (n = 10 per trial) mice were released into 0.056 hectare (38.1 m x 15.24 m) enclosures for 10-day observation periods before they were recovered using live-trapping methods (C57 trials: n = 4; outbred trials: n = 3). The walls of the enclosures were made from sheet metal and stood approximately 4 feet tall and extended 4 feet into the ground to prevent the mice from tunneling and moving between the enclosures. Each enclosure was covered with netting to prevent aerial predation, and loose gravel was spread along the interior perimeter of each enclosure to discourage digging near the walls. Three days prior to releasing mice into the enclosures, we trapped in and around the enclosures to capture and remove any small mammals or snakes from the enclosure. The enclosures contained a mixture of local perennial grasses and plant communities which were mowed to a height of ~5 cm prior at the start of each trial.

Each enclosure contained eight identical resource zones constructed of PVC and 32-gallon storage totes (Rubbermaid, USA) arranged in a two by four grid pattern. Resource zones were covered with waterproof corrugated roofing material attached to a polyvinyl chloride (PVC) frame. Resource zones had a single PVC entrance tube (50mm diameter) through which the mice could freely enter or exit the tub. Each resource zone contained feeder towers containing food and water in excess (~50 grams of sunflower seed and 2 liters of water). Several pieces of plastic lumber were added to provide environmental complexity and vantage points for the mice. The interior of each resource zone was monitored by a single motion-activated infrared camera with a 180-degree field of view (HD-Q3, CCTV

Camera Pros, Lantana, FL, USA) connected to a central DVR unit for file storage and data offloading. Additionally, each resource zone was equipped with a 15 cm RFID antenna connected to a centralized data acquisition unit (Biomark, Small Scale System, Boise, ID, USA). Antennas were placed directly beneath the floor adjacent to the PVC zone entrance tubes to increase the likelihood of capturing mouse entrances and exits from the resource zone. Scanning for RFID tags within the antenna range occurred at approximately 2-3 Hz continuously for 10 days. At least 24 hours prior to release in the enclosures, all subjects were placed into a stereotaxic frame (Kopf Instruments, Tuhunga, CA, USA) and briefly anesthetized with isoflurane (3-5%). Mice were subcutaneously implanted with dual RFID tags (BioMark, Boise, ID, USA) in the dorsal flank and periscapular region.

At the conclusion of the 10-day observation period, the resource zone entrance tubes were blocked and >50 live-catch traps (H.B. Sherman, Tallahassee, FL, USA) baited with sunflower seeds and a moistened cotton ball were placed in a grid pattern in the enclosures in the evening (20:00-22:00 hours) and were checked for occupancy the following morning (07:00-09:00 hours). Trapping continued until all the mice were recovered or identified as deceased or missing (a conclusion reached if there were no RFID reads in the enclosure for a 24-hour period after 3 days of trapping). The trap locations were recorded, and the individual identities of the mice were confirmed using a handheld RFID reader (BioMark, HPR Lite).

RFID data analysis and zone visit estimation

We examined the time elapsed between consecutive RFID detection events for each mouse within each resource zone (the RFID inter-read interval). We found that the distribution of all RFID inter-read intervals was heavily skewed (min = 1s, median = 1s, mean = 16.4s, max = 32,683s). We grouped RFID reads into zone visitation bouts using a 153 second (the cut-off for capturing 99% of all the mouse inter-read interval values) sliding window method. Based on visual observations of the resource zones and on RFID data, we omitted a subset of animals from a subset of days for all spatial and social analyses (see Table S1 for details).

Priority Access Score calculation

Priority access scores were calculated separately for male and female mice within a trial. First, we calculated the time a given mouse (*M*) occupied a resource zone (*Z*) as a percentage of the total time that zone was occupied by same-sex conspecifics on a given day (*D*).

$$Occupancy_{M,D,Z} = \frac{time_{M,D,Z}}{\sum_{m=1}^{10} time_{m,D,Z}}$$

Next, we calculated a daily Capture Score by summing the Occupancy values for all available zones. Mice that did not have an occupancy value of greater than 0.5 (in other words, a majority share of the

time spent in any particular zone), were penalized by subtracting 1 from the final Capture Score. The penalty indicates that on a given day, a mouse failed to capture any of the zones that mouse visited.

$$Capture\ Score_{M,D} = \begin{cases} \sum_{z=1}^{8} Occupancy_{M,D,z} - 1 & \text{if } \exists\ Occupancy_{M,D,z} < 0.5\ \forall\ z = 1, \dots, 8 \\ \sum_{z=1}^{8} Occupancy_{M,D,z} & otherwise \end{cases}$$

To see how access to zones changed over time, we took the cumulative sum of an individual's Capture Score ordinally across each day of the trial to derive a final Priority Access Score.

$$Priority\ Access\ Score_{M,D} = \sum_{d=1}^{D} Capture\ Score_{M,d}$$

As an example, if one male (Male A) occupied a single resource zone every day of the trial for 4 hours a day, while another male (Male B) accessed only that same zone for 1 hour per day, and the zone was visited by no other mice, each mouse would yield the following values. Male A's daily Capture Score would equal 0.8, (because he controlled 4 out of 5 hours), while Male B's daily Capture score would equal -0.8 (because he controlled 1 out of 5 hours and received a one-point penalty for not controlling any zones). If this pattern of visitation remained unchanged for all 10 days, then Male A's final PAS would equal 8, while Male B's PAS would equal -8. Thus, a mouse that is the sole, uncontested occupant of 3 independent zones (for any length of time) repeatedly over the course of 10 days would have a daily Capture Score of 3, and a final PAS value of 30. The PAS value thus provides a temporally evolving measure that captures the dynamics of territory formation, maintenance, and collapse (**Fig. S2c-d**).

Social Networks

Weighted networks were derived from a Simple Ratio Index calculation based on binary participation in spatially and temporally overlapping mouse grouping events in the resource zones using the *asnipe*[90] package in R 4.1.2 (R Development Core Team). As individuals' social networks may be affected by the size of the social group, we omitted a subset of individuals who had limited data due to death or loss of RFID chips[91,92] (see **Table S1**).

Statistical Analyses

We built mixed effects models using R 4.1.2 (R Development Core Team) and the R packages *lme4*[93], *lmerTest*[94], and *emmeans*[95] to examine relationships between predictor and response variables. We included relevant random intercepts and random slopes in our models as appropriate. When main

effects or interaction effects achieved statistical significance ($P < 0.05$, two-tailed), we performed *post-hoc* univariate ANOVAs. We only report significant main and interaction effects that are critical for data interpretation from our multifactorial ANOVAs in the Results section. We include the full statistical test and model outputs in the Supplementary Material. Data cleaning, shaping and summaries were performed in R. Graphing was performed in R using the package *ggplot2*[96] and in GraphPad Prism 9.3 (www.graphpad.com). We report all means ± standard error measure (SEM), unless otherwise stated, and consider all values statistically significant when $P < 0.05$.

464 **SUPPLEMENTARY FIGURES**

	C57 Trials (10 days per trial)				Outbred Trials (10 days per trial)			Total
	Trial 1	**Trial 2**	**Trial 3**	**Trial 6**	**Trial 4**	**Trial 5**	**Trial 7**	
Strain	C57	C57	C57	C57	Outbred	Outbred	Outbred	-
Enclosure	Bravo	Alpha	Bravo	Alpha	Alpha	Bravo	Bravo	-
Trial Dates	6/19/2020-6/29/2020	7/3/2020 - 7/13/2020	7/3/2020 - 7/13/2020	8/13/2020 - 8/23/2020	7/17/2020 - 7/27/2020	7/17/2020 - 7/27/2020	8/13/2020 - 8/23/2020	-
Total RFID Reads	1,387,206	1,435,575	903,013	1,052,007	1,140,586	1,579,413	890,836	8,388,636
Average RFID reads per mouse per night	6936	7178	5017	5260	6631	7897	4454	-
Total estimated mouse hours spent in resource zones	F: 406.9 M: 503.7	F: 328.9 M: 521.9	F: 328.9 M: 521.9	F: 530.0 M: 524.4	F: 494.4 M: 380.6	F: 284.2 M: 526.0	F: 360.1 M: 290.3	5833.3
Mice Collected / Released	20 / 20	20 / 20	20 / 20	20 / 20	18 / 20	20 / 20	20 / 20	
Triage Details C = collected ND = not detected NC = not collected PD = presumed dead	NA	NA	- Anubis (male) dead on Day 5, C - Rae (female) C, ND Day 2 – 10 - Rose (female) ND on Day 10, trapped without RFID tags	NA	- Hare (male) ND Day 2 – 10, NC, PD - Isis (female) ND Day 3 – 10, NC, PD - George (male) crosses from Trial 4 to Trial 5 paddock on Day 3, C. Triaged from all analyses.	NA	NA	

465
466 **Table S1: Summary of trial details.**
467

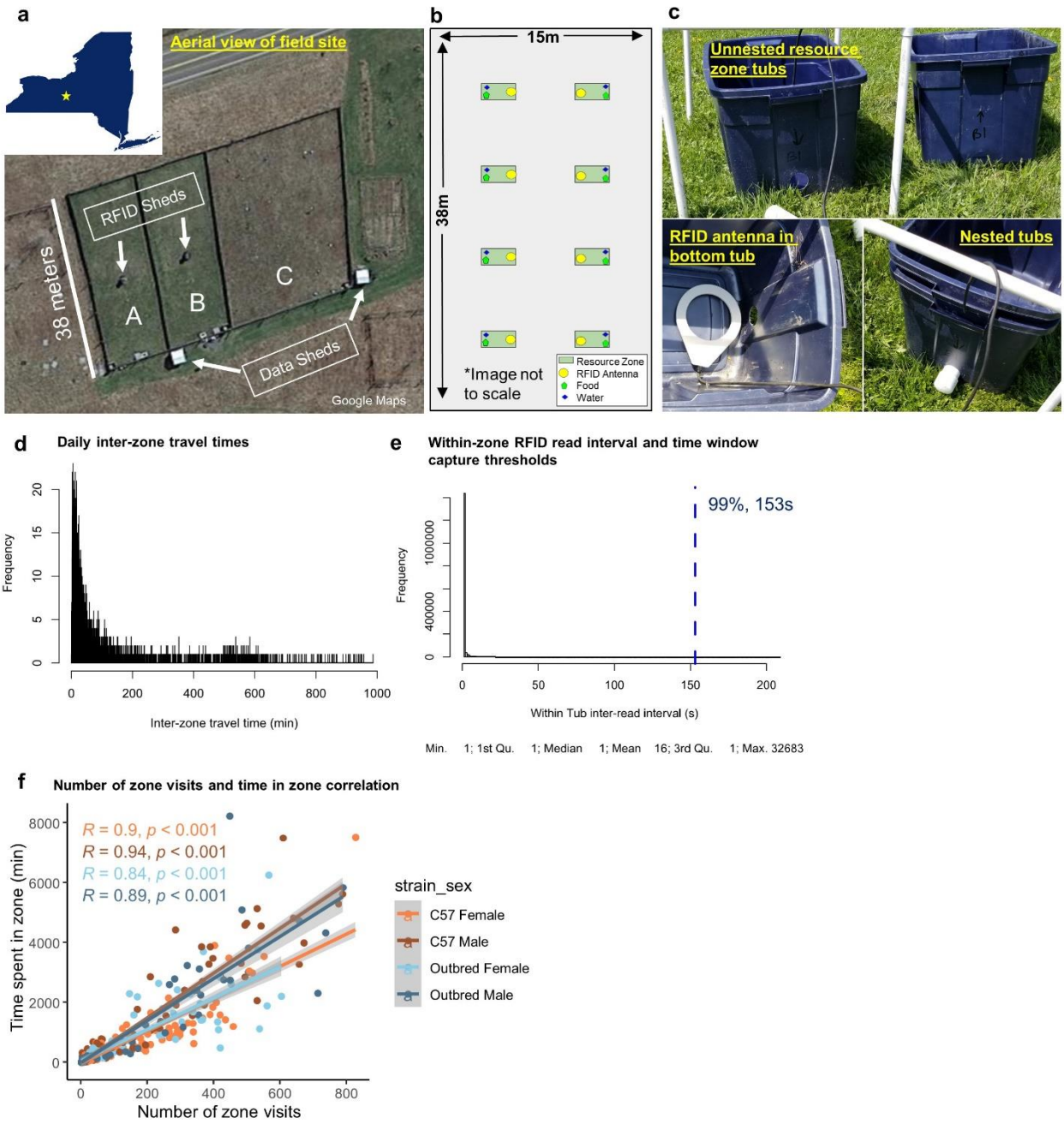

Figure S1: Field site setup and RFID duration bout window selection. (a) Satellite image of the field enclosures showing the position of the data sheds housing the security camera system and computer for downloading RFID data from the central RFID sheds. **(b)** Schematic of the Alpha and Bravo enclosures indicating the resource zone layouts. **(c)** RFID monitoring of the resource zones. Two storage totes were nested with a RFID antenna placed between and beneath the entrance tunnel to prevent mice from directly contacting the antenna and wire. **(d)** Histogram of the daily inter-zone travel times for all mice for all days. **(e)** Histogram of the within zone inter- RFID read intervals and the 153 second threshold capturing 99% of all inter-RFID read intervals which was used to group RFID reads into resource zone visitation bouts (see Methods). **(f)** Correlation of estimated duration spent in each zone and the number of visits to that zone for all sex and strain categories.

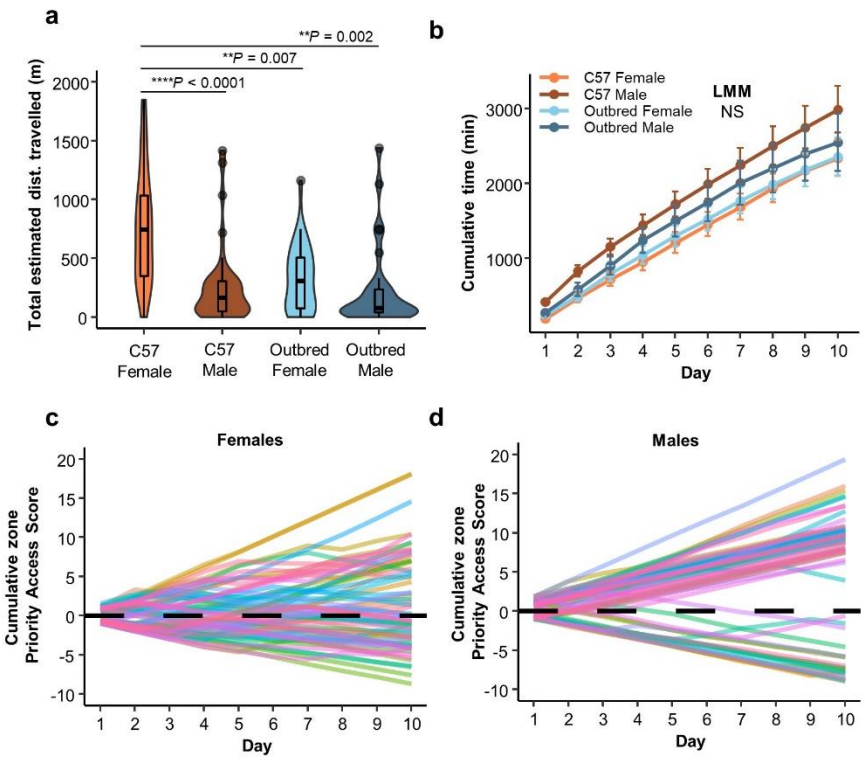

Figure S2: Estimated time in resource zones and priority access score development. (a) Total estimated distance travelled across the trial period for all sex and genotype categories. **(b)** Cumulative sum of daily estimated time spent in the resource zones. **(c-d)** Cumulative sum of daily Priority Access scores over 10 days of observation for females **(c)** and males **(d)** of both genotypes (See Methods for additional details on calculation of the daily PAS value).

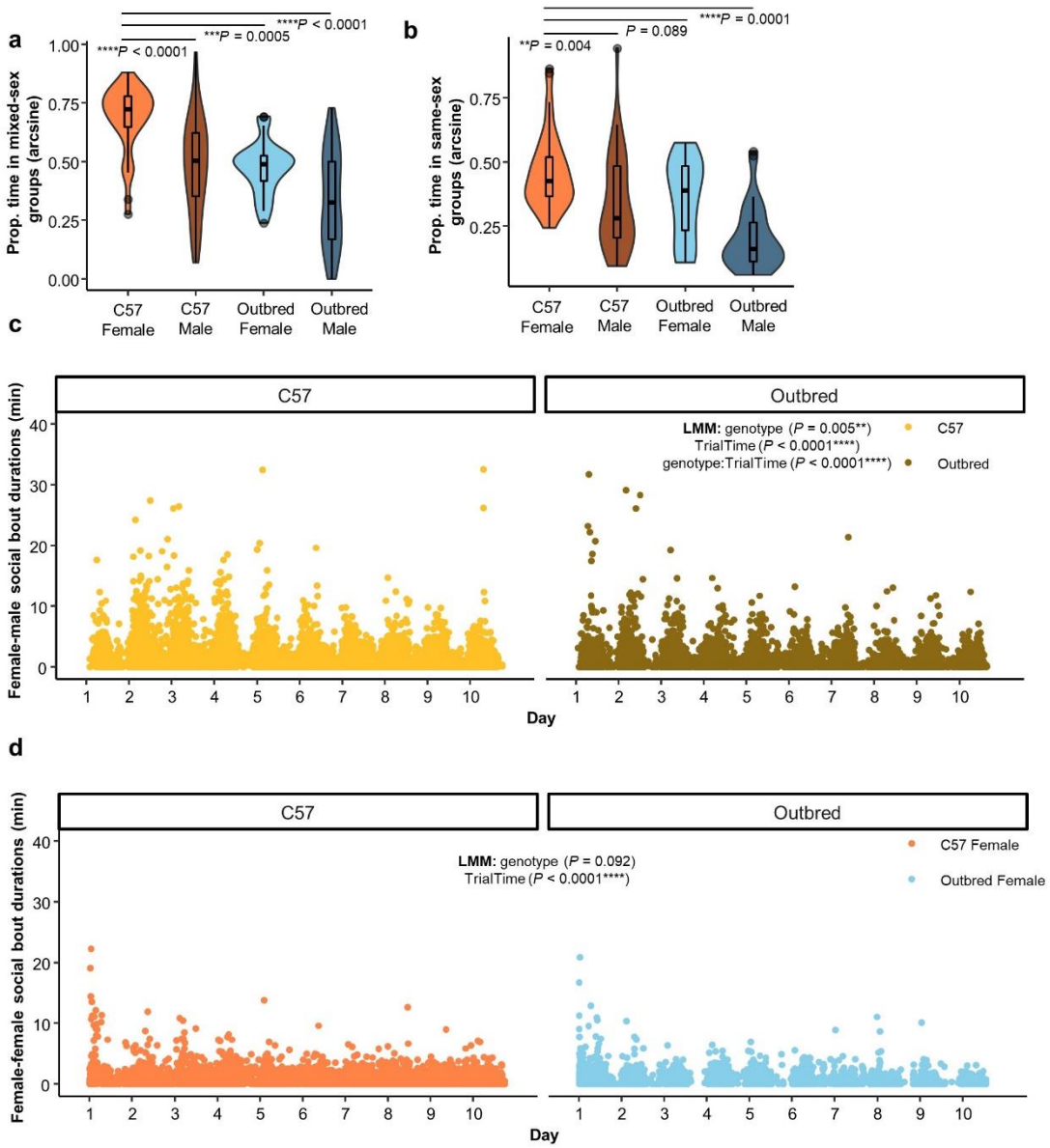

Figure S3: Sex and genotype social grouping compositions. (a) Proportion of mouse time spent in mixed sex groups. **(b)** Proportion of time spent in same sex groups. **(c)** Female-male social grouping bout durations over time. For visualization purposes, the y-axis is cut off at 40 (n = 19,736 events shown out of 19,738 total events). **(d)** Female-female social grouping bout durations over time. For visualization purposes, the y-axis is cut off at 40 (n = 9,160 events shown out of 9,161 total events).

474

475

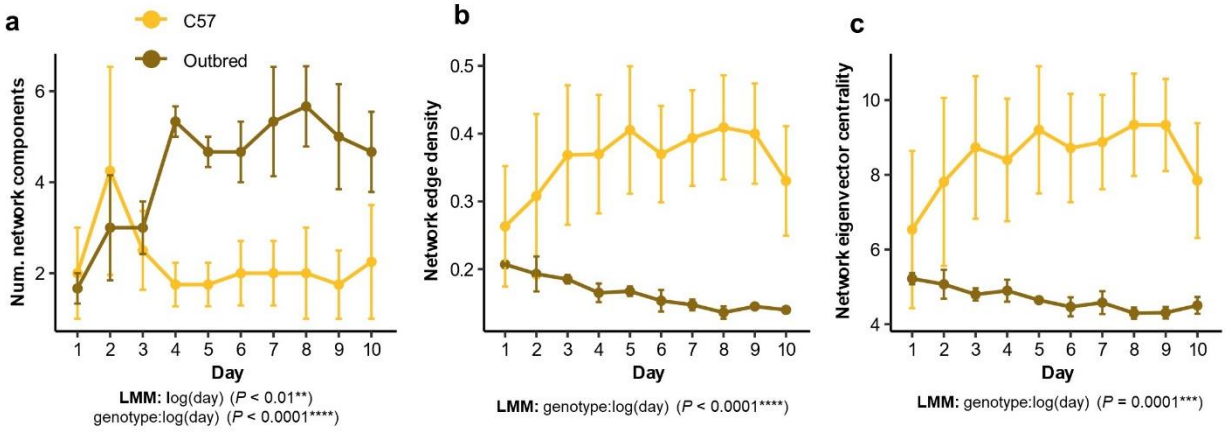

Figure S4: C57 and outbred social network-level properties over time. (a) Number of network components increases over time in outbred, but not C57, social networks over time. **(b)** Network edge density increases over time in C57, but not outbred, social networks over time. **(c)** Network eigenvector centrality significantly differs between C57 and outbred networks over time.

476

477

REFERENCES

1. Fox, J.G., Barthold, S., Davisson, M., Newcomer, C.E., Quimby, F.W., and Smith, A. (2006). The mouse in biomedical research: normative biology, husbandry, and models (Elsevier).

2. Rosenthal, N., and Brown, S. (2007). The mouse ascending: perspectives for human-disease models. Nat Cell Biol *9*, 993–999.

3. Snell, G.D. (1941). Biology of the laboratory mouse.

4. Waterston, R.H., and Pachter, L. (2002). Initial sequencing and comparative analysis of the mouse genome. Nature *420*, 520–562.

5. Paigen, K. (2003). One Hundred Years of Mouse Genetics: An Intellectual History. I. The Classical Period (1902-1980). Genetics *163*, 1–7.

6. Paigen, K. (2003). One Hundred Years of Mouse Genetics: An Intellectual History. II. The Molecular Revolution (1981-2002). Genetics *163*, 1227–1235.

7. Graham, A.L. (2021). Naturalizing mouse models for immunology. Nat Immunol *22*, 111–117.

8. Lahvis, G.P. (2017). Unbridle biomedical research from the laboratory cage. eLife *6*, e27438.

9. Leonelli, S., and Ankeny, R.A. (2013). What makes a model organism? Endeavour *37*, 209–212.

10. Datta, S.R., Anderson, D.J., Branson, K., Perona, P., and Leifer, A. (2019). Computational Neuroethology: A Call to Action. Neuron *104*, 11–24.

11. Dennis, E.J., Hady, A.E., Michaiel, A., Clemens, A., Tervo, D.R.G., Voigts, J., and Datta, S.R. (2021). Systems Neuroscience of Natural Behaviors in Rodents. J. Neurosci. *41*, 911–919.

12. Gomez-Marin, A., Paton, J.J., Kampff, A.R., Costa, R.M., and Mainen, Z.F. (2014). Big behavioral data: psychology, ethology and the foundations of neuroscience. Nat Neurosci *17*, 1455–1462.

13. Krakauer, J.W., Ghazanfar, A.A., Gomez-Marin, A., Maciver, M.A., and Poeppel, D. (2017). Neuroscience Needs Behavior: Correcting a Reductionist Bias. Neuron *93*, 480–490.

14. Laurent, G. (2020). On the value of model diversity in neuroscience. Nat Rev Neurosci *21*, 395–396.

15. Pereira, T.D., Shaevitz, J.W., and Murthy, M. (2020). Quantifying behavior to understand the brain. Nat Neurosci *23*, 1537–1549.

16. Zilkha, N., Sofer, Y., Beny, Y., and Kimchi, T. (2016). From classic ethology to modern neuroethology: Overcoming the three biases in social behavior research. Current Opinion in Neurobiology.

17. Torquet, N., Marti, F., Campart, C., Tolu, S., Nguyen, C., Oberto, V., Benallaoua, M., Naudé, J., Didienne, S., Debray, N., et al. (2018). Social interactions impact on the dopaminergic system and drive individuality. Nature Communications *9*, 3081.

18. Weissbrod, A., Shapiro, A., Vasserman, G., Edry, L., Dayan, M., Yitzhaky, A., Hertzberg, L., Feinerman, O., and Kimchi, T. (2013). Automated long-term tracking and social behavioural phenotyping of animal colonies within a semi-natural environment. Nat Commun *4*, 2018.

19. Williamson, C.M., Lee, W., and Curley, J.P. (2016). Temporal dynamics of social hierarchy formation and maintenance in male mice. Animal Behaviour *115*, 259–272.

20. Bronson, F.H. (1979). REPRODUCTIVE ECOLOGY OF THE HOUSE MOUSE. Quarterly Review of Biology *54*, 265–299.

21. Hurst, J.L. (1987). The functions of urine marking in a free-living population of house mice, Mus domesticus Rutty. Animal Behaviour *35*, 1433–1442.

22. König, B., Lindholm, A.K., Lopes, P.C., Dobay, A., Steinert, S., and Buschmann, F.J.-U. (2015). A system for automatic recording of social behavior in a free-living wild house mouse population. Animal Biotelemetry *3*, 39.

23. Pocock, M.J.O., Searle, J.B., and White, P.C.L. (2004). Adaptations of animals to commensal habitats: population dynamics of house mice Mus musculus domesticus on farms. Journal of Animal Ecology *73*, 878–888.

24. Pocock, M.J.O., Hauffe, H.C., and Searle, J.B. (2005). Dispersal in house mice. Biological Journal of the Linnean Society *84*, 565–583.

25. Crowcroft, P. (1966). Mice All Over.

26. Crowcroft, P., and Rowe, F.P. (1963). Social Organisation and Territorial Behaviour in the Wild House Mouse (Mus musculus L.). Proceedings of the Zoological Society of London *140*, 517–531.

27. Gerlach, G. (1996). Emigration mechanisms in fetal house mice - A laboratory investigation of the influence of social structure, population density, and aggression. Behavioral Ecology and Sociobiology *39*, 159–170.

28. Lidicker, W.Z. (1976). Social Behaviour and Density Regulation in House Mice Living in Large Enclosures. Journal of Animal Ecology *45*, 677–697.

29. Luzynski, K.C., Nicolakis, D., Marconi, M.A., Zala, S.M., Kwak, J., and Penn, D.J. (2021). Pheromones that correlate with reproductive success in competitive conditions. Sci Rep *11*, 21970.

30. Meagher, S., Penn, D.J., and Potts, W.K. (2000). Male–male competition magnifies inbreeding depression in wild house mice. PNAS *97*, 3324–3329.

31. Noyes, R.F., Barrett, G.W., and Taylor, D.H. (1982). Social Structure of Feral House Mouse (Mus musculus L.) Populations: Effects of Resource Partitioning. Behavioral Ecology and Sociobiology *10*, 157–163.

32. Potts, W.K., Manning, C.J., and Wakeland, E.K. (1991). Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature *352*, 619–621.

33. Sherborne, A.L., Thom, M.D., Paterson, S., Jury, F., Ollier, W.E.R., Stockley, P., Beynon, R.J., and Hurst, J.L. (2007). The genetic basis of inbreeding avoidance in house mice. Current Biology *17*, 2061–2066.

34. Stockley, P., Ramm, S.A., Sherborne, A.L., Thom, M.D., Paterson, S., and Hurst, J.L. (2013). Baculum morphology predicts reproductive success of male house mice under sexual selection. BMC biology *11*, 1–6.

35. Wolff, R.J. (1985). Mating behaviour and female choice: their relation to social structure in wild caught House mice (Mus musculus) housed in a semi-natural environment. Journal of Zoology *207*, 43–51.

36. Crowcroft, P. (1955). Territoriality in Wild House Mice, Mus musculus L. Journal of Mammalogy *36*, 299–301.

37. Brown, R.Z. (1953). Social Behavior, Reproduction, and Population Changes in the House Mouse (Mus musculus L.). Ecological Monographs *23*, 218–240.

38. Palanza, P., Re, L., Mainardi, D., Brain, P.F., and Parmigiani, S. (1996). Male and female competitive strategies of wild house mice pairs (Mus musculus domesticus) confronted with intruders of different sex and age in artificial territories. Behaviour *133*, 863–882.

39. Palanza, P., Della Seta, D., Ferrari, P.F., and Parmigiani, S. (2005). Female competition in wild house mice depends upon timing of female/male settlement and kinship between females. Animal Behaviour *69*, 1259–1271.

40. Hurst, J.L. (1987). Behavioural variation in wild house mice Mus domesticus Rutty: A quantitative assessment of female social organization. Animal Behaviour *35*, 1846–1857.

41. Chovnick, A., Yasukawa, N.J., Monder, H., and Christian, J.J. (1987). Female behavior in populations of mice in the presence and absence of male hierarchy. Aggressive Behavior *13*, 367–375.

42. Chalfin, L., Dayan, M., Levy, D.R., Austad, S.N., Miller, R.A., Iraqi, F.A., Dulac, C., and Kimchi, T. (2014). Mapping ecologically relevant social behaviours by gene knockout in wild mice. Nat Commun *5*, 4569.

43. Justice, M.J., and Dhillon, P. (2016). Using the mouse to model human disease: increasing validity and reproducibility. Disease Models & Mechanisms *9*, 101–103.

44. Nelson, A.C., Cauceglia, J.W., Merkley, S.D., Youngson, N.A., Oler, A.J., Nelson, R.J., Cairns, B.R., Whitelaw, E., and Potts, W.K. (2013). Reintroducing domesticated wild mice to sociality induces adaptive transgenerational effects on MUP expression. Proc. Natl. Acad. Sci. U.S.A. *110*, 19848–19853.

45. Perlman, R.L. (2016). Mouse models of human disease: An evolutionary perspective. Evolution, Medicine, and Public Health *2016*, 170–176.

579 46. Worp, H.B. van der, Howells, D.W., Sena, E.S., Porritt, M.J., Rewell, S., O'Collins, V., and
580 Macleod, M.R. (2010). Can Animal Models of Disease Reliably Inform Human Studies? PLOS
581 Medicine *7*, e1000245.

582 47. Cope, E.C., Opendak, M., LaMarca, E.A., Murthy, S., Park, C.Y., Olson, L.B., Martinez, S., Leung,
583 J.M., Graham, A.L., and Gould, E. (2019). The effects of living in an outdoor enclosure on
584 hippocampal plasticity and anxiety-like behavior in response to nematode infection. Hippocampus
585 *29*, 366–377.

586 48. Daan, S., Spoelstra, K., Albrecht, U., Schmutz, I., Daan, M., Daan, B., Rienks, F., Poletaeva, I.,
587 Dell'Omo, G., and Vyssotski, A. (2011). Lab mice in the field: unorthodox daily activity and effects
588 of a dysfunctional circadian clock allele. Journal of biological rhythms *26*, 118–129.

589 49. Leung, J.M., Budischak, S.A., The, H.C., Hansen, C., Bowcutt, R., Neill, R., Shellman, M., Loke,
590 P., and Graham, A.L. (2018). Rapid environmental effects on gut nematode susceptibility in
591 rewilded mice. PLOS Biology *16*, e2004108.

592 50. Lin, J.-D., Devlin, J.C., Yeung, F., McCauley, C., Leung, J.M., Chen, Y.-H., Cronkite, A., Hansen,
593 C., Drake-Dunn, C., Ruggles, K.V., et al. (2020). Rewilding Nod2 and Atg16l1 Mutant Mice
594 Uncovers Genetic and Environmental Contributions to Microbial Responses and Immune Cell
595 Composition. Cell Host & Microbe *27*, 830-840.e4.

596 51. Yeung, F., Chen, Y.-H., Lin, J.-D., Leung, J.M., McCauley, C., Devlin, J.C., Hansen, C., Cronkite,
597 A., Stephens, Z., Drake-Dunn, C., et al. (2020). Altered Immunity of Laboratory Mice in the Natural
598 Environment Is Associated with Fungal Colonization. Cell Host & Microbe *27*, 809-822.e6.

599 52. Filazzola, A., and Cahill Jr, J.F. (2021). Replication in field ecology: Identifying challenges and
600 proposing solutions. Methods in Ecology and Evolution *12*, 1780–1792.

601 53. Fraser, H., Barnett, A., Parker, T.H., and Fidler, F. (2020). The role of replication studies in ecology.
602 Ecology and Evolution *10*, 5197–5207.

603 54. Anderson, P.K. (1961). Density, social structure, and nonsocial environment in house-mouse
604 populations and the implications for regulation of numbers. Transactions of the New York Academy
605 of Sciences *23*, 447–451.

606 55. Guo, M., Wu, C.F., Liu, W., Yang, J.Y., and Chen, D. (2004). Sex difference in psychological
607 behavior changes induced by long-term social isolation in mice. Progress in Neuro-
608 Psychopharmacology and Biological Psychiatry *28*, 115–121.

609 56. Koike, H., Ibi, D., Mizoguchi, H., Nagai, T., Nitta, A., Takuma, K., Nabeshima, T., Yoneda, Y., and
610 Yamada, K. (2009). Behavioral abnormality and pharmacologic response in social isolation-reared
611 mice. Behavioural brain research *202*, 114–121.

612 57. Matthews, G.A., Nieh, E.H., Vander Weele, C.M., Halbert, S.A., Pradhan, R.V., Yosafat, A.S.,
613 Glober, G.F., Izadmehr, E.M., Thomas, R.E., Lacy, G.D., et al. (2016). Dorsal Raphe Dopamine
614 Neurons Represent the Experience of Social Isolation. Cell *164*.

58. Nonogaki, K., Nozue, K., and Oka, Y. (2007). Social isolation affects the development of obesity and type 2 diabetes in mice. Endocrinology *148*, 4658–4666.

59. Zelikowsky, M., Hui, M., Karigo, T., Choe, A., Yang, B., Blanco, M.R., Beadle, K., Gradinaru, V., Deverman, B.E., and Anderson, D.J. (2018). The Neuropeptide Tac2 Controls a Distributed Brain State Induced by Chronic Social Isolation Stress. Cell *173*, 1265-1279.e19.

60. Kaufmann, J.H. (1983). On the Definitions and Functions of Dominance and Territoriality. Biological Reviews *58*, 1–20.

61. Pyke, G.H. (1979). The Economics of Territory Size and Time Budget in the Golden-Winged Sunbird. The American Naturalist *114*, 131–145.

62. Stamps, J. (1994). Territorial Behavior: Testing the Assumptions. Advances in the Study of Behavior *23*, 173–232.

63. Chambers, L.K., Singleton, G.R., and Krebs, C. (2000). Movements and Social Organization of Wild House Mice (Mus Domesticus) in the Wheatlands of Northwestern Victoria , Australia. Journal of Mammalogy *81*, 59–69.

64. Singleton, G.R. (1983). The Social and Genetic Structure of a Natural Colony of House Mice, Mus musculus, at Healesville WildlifeSanctuary. Aust. J. Zool. *31*, 155–166.

65. Barton, R.A., Byrne, R.W., and Whiten, A. (1996). Ecology, feeding competition and social structure in baboons. Behavioral Ecology and Sociobiology *38*, 321–329.

66. Faulkes, C.G., Bennett, N.C., Bruford, M.W., Obrien, H.P., Aguilar, G.H., and Jarvis, J.U.M. (1997). Ecological constraints drive social evolution in the African mole-rats. Proceedings of the Royal Society of London Series B-Biological Sciences *264*, 1619–1627.

67. Lukas, D., and Clutton-Brock, T. Climate and the distribution of cooperative breeding in mammals. Royal Society Open Science *4*, 160897.

68. Elliser, C.R., and Herzing, D.L. (2014). Social structure of Atlantic spotted dolphins, Stenella frontalis, following environmental disturbance and demographic changes. Marine Mammal Science *30*, 329–347.

69. Ostfeld, R.S., Lidicker, W.Z., and Heske, E.J. (1985). The Relationship between Habitat Heterogeneity, Space Use, and Demography in a Population of California Voles. Oikos *45*, 433–442.

70. Palombit, R.A., Seyfarth, R.M., and Cheney, D.L. (1997). The adaptive value of 'friendships' to female baboons: experimental and observational evidence. Animal Behaviour *54*, 599–614.

71. Evans, J.C., Liechti, J.I., Boatman, B., and König, B. (2020). A natural catastrophic turnover event: individual sociality matters despite community resilience in wild house mice. Proceedings of the Royal Society B: Biological Sciences *287*, 20192880.

649 72. Lukas, D., and Clutton-Brock, T. (2012). Cooperative breeding and monogamy in mammalian
650 societies. Proceedings of the Royal Society B-Biological Sciences *279*, 2151–2156.

651 73. Dobson, F.S., Way, B.M., and Baudoin, C. (2010). Spatial dynamics and the evolution of social
652 monogamy in mammals. Behavioral Ecology *21*, 747–752.

653 74. Perez-Barberia, F.J., Shultz, S., and Dunbar, R.I.M. (2007). Evidence for coevolution of sociality
654 and relative brain size in three orders of mammals. Evolution *61*, 2811–2821.

655 75. Schradin, C. (2013). Intraspecific variation in social organization by genetic variation,
656 developmental plasticity, social flexibility or entirely extrinsic factors. Philosophical Transactions of
657 the Royal Society B: Biological Sciences *368*, 20120346.

658 76. Schradin, C., Hayes, L.D., Pillay, N., and Bertelsmeier, C. (2018). The evolution of intraspecific
659 variation in social organization. Ethology *124*, 527–536.

660 77. Li, S.W., Zeliger, O., Strahs, L., Báez-Mendoza, R., Johnson, L.M., McDonald Wojciechowski, A.,
661 and Williams, Z.M. (2022). Frontal neurons driving competitive behaviour and ecology of social
662 groups. Nature *603*, 661–666.

663 78. Clutton-Brock, T. (2021). Social evolution in mammals. Science *373*, eabc9699.

664 79. König, B., Lindholm, A.K., Macholan, M., Baird, S.J., Munclinger, P., and Pialek, J. (2012). The
665 complex social environment of female house mice (Mus domesticus). Cambridge studies in
666 morphology and molecules: new paradigms in evolutionary bio, 114–134.

667 80. Wolff, J.O. (1993). Why are female small mammals territorial? Oikos, 364–370.

668 81. Wolff, J.O. (2007). Social biology of rodents. Integrative Zoology *2*, 193–204.

669 82. Rowe, F.P., and Redfern, R. (1969). Aggressive behaviour in related and unrelated wild house mice
670 (Mus musculus L.). Annals of Applied Biology *64*, 425–431.

671 83. Barnard, C.J., and Fitzsimons, J. (1989). Kin recognition and mate choice in mice: fitness
672 consequences of mating with kin. Animal Behaviour *38*, 35–40.

673 84. Krackow, S., and Matuschak, B. (1991). Mate Choice for Non-siblings in Wild House Mice:
674 Evidence from a Choice Test and a Reproductive Test. Ethology *88*, 99–108.

675 85. Lee, W., Milewski, T.M., Dwortz, M.F., Young, R.L., Gaudet, A.D., Fonken, L.K., Champagne,
676 F.A., and Curley, J.P. (2021). Distinct inflammatory and transcriptomic profiles in dominant versus
677 subordinate males in mouse social hierarchies. 2021.09.04.458987.

678 86. Phifer-Rixey, M., and Nachman, M.W. (2015). The natural history of model organisms: insights into
679 mammalian biology from the wild house mouse Mus musculus. Elife *4*, e05959.

87. Phifer-Rixey, M., Bi, K., Ferris, K.G., Sheehan, M.J., Lin, D., Mack, K.L., Keeble, S.M., Suzuki, T.A., Good, J.M., and Nachman, M.W. (2018). The genomic basis of environmental adaptation in house mice. PLoS Genetics *14*, e1007672.

88. Hatchwell, B.J. (2017). Replication in behavioural ecology: a comment on Ihle et al. Behavioral Ecology *28*, 360–360.

89. Kelly, C.D. (2006). Replicating empirical research in behavioral ecology: how and why it should be done but rarely ever is. The Quarterly review of biology *81*, 221–236.

90. Farine, D.R. (2013). Animal social network inference and permutations for ecologists in R using asnipe. Methods in Ecology and Evolution *4*, 1187–1194.

91. Aplin, L.M., Firth, J.A., Farine, D.R., Voelkl, B., Crates, R.A., Culina, A., Garroway, C.J., Hinde, C.A., Kidd, L.R., Psorakis, I., et al. (2015). Consistent individual differences in the social phenotypes of wild great tits, Parus major. Animal Behaviour *108*, 117–127.

92. Farine, D.R., and Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. Journal of Animal Ecology *84*, 1144–1163.

93. Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4.

94. Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software *82*, 1–26.

95. Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. Journal of Statistical Software *69*, 1–33.

96. Wickham, H. (2016). Programming with ggplot2. In ggplot2: Elegant Graphics for Data Analysis Use R!, H. Wickham, ed. (Springer International Publishing), pp. 241–253.