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Review

Hormones, life-history, and phenotypic variation: Opportunities in evolutionary avian endocrinology

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ABSTRACT

Life-histories provide a powerful, conceptual framework for integration of endocrinology, evolutionary biology and ecology. This has been a commonly articulated statement but here I show, in the context of avian reproduction, that true integration of ultimate and proximate approaches has been slow. We have only a rudimentary understanding of the physiological and hormonal basis of *phenotypic* variation in (a) reproductive traits that contribute most to individual variation in lifetime fitness in birds (e.g. laying date, clutch size, parental effort) and (b) trade-offs that link these traits or that link reproduction to other life stages (e.g. migration, molt). I suggest that some reasons for this relative lack of progress include (a) an increasingly reductionist and centralist (upstream) focus which is more and more removed from ecological/evolutionary context, and from peripheral (downstream) mechanisms that actually determine how phenotypes work (b) a long-standing male-bias in experimental studies, even though the key reproductive traits which contribute most to variation in fitness are female-specific traits (e.g. onset of vitellogenesis, egg size or number). Endocrine systems provide strong candidate mechanisms for regulation of phenotypic variation in single traits, and two endocrine concepts capture the essence of life-history trade-offs: (a) hormonal 'pleiotropy', when single hormones have both positive and negative effects on multiple physiological systems and (b) hormonal conflict between regulatory systems required for different but over-lapping or linked life-history stages. I illustrate these ideas with examples of reproductive anemia, migration-reproduction overlap, and molt-breeding overlap, to highlight some of the tremendous opportunities that exist for *comparative endocrinologists* to contribute to mechanistic studies of avian reproduction in an evolutionary context.

> "We have for the most part contented ourselves with the figure of one sex only, and that the male"
> John Ray (1676, cited in [40])

1. Introduction

What do we know about the hormonal mechanisms underlying individual, phenotypic variation in the key reproductive life-history traits of birds? This might seem an odd question given that integration of endocrinology with ecology, and more recently with evolutionary biology, has been a commonly articulated theme over the last 20–30 years [52,53,106–108]. However, true integration of ultimate and proximate approaches has been slow in many areas of organismal 'evolutionary physiology' [15,50]. Here, and elsewhere [102], I argue that this is particularly the case in the context of *female* reproduction in birds. Specifically, we have only a very rudimentary, and in some cases non-existent, understanding of the mechanisms underlying (a) individual, phenotypic, variation in key life-history traits such as timing of breeding, clutch size and parental care and (b) trade-offs or carry-over effects between these traits, and between reproduction and other life-history stages (e.g. migration, molt). In this review I will firstly illustrate some of the reasons for this lack of progress using timing of breeding, clutch size and parental care as examples. I will then provide examples of two concepts, hormonal 'pleiotropy' and hormonal or physiological 'conflict', which I think capture the essence of evolutionary or life-history trade-offs. My main goal is to highlight some of the tremendous opportunities these knowledge gaps present for comparative endocrinologists to contribute to mechanistic studies of avian reproduction in an evolutionary context.

2. Which traits are important, and why study phenotypic variation in females?

Before considering some specific examples I want to deal with two general, and related, issues: (a) how we should identify or define the "important" reproductive traits that should be our

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research focus and (b) why we should work on *female* reproductive traits and individual or phenotypic variation. In the context of evolutionary endocrinology I would argue that we need to identify hormonal mechanisms for reproductive traits that contribute most to variance in life-time *fitness*. An increasing number of long-term, individual-based, population studies of birds and mammals are providing answers to this question, measuring empirical differences in fitness between individuals and assessing the causes of these differences [19]. These studies show that the traits most strongly correlated with individual variation in lifetime fitness (estimated as the total number of offspring *recruited* to the breeding population) include timing of egg-laying, clutch size, longevity, and the total number of young fledged from all breeding attempts, which is a composite of how well individuals look after their offspring, i.e. parental care [17,38,63,82]. Longevity is outside the scope of this review but, in the context of ageing and senescence, represents another fascinating area of integration of mechanism and evolutionary biology [66,67] Two of these "key" reproductive life-history traits, timing of egg-laying and clutch size, are clearly *female-specific* traits. Both sexes can contribute, sometimes equally, to parental care but even for this stage of breeding sex-specific differences in parental care are routinely attributed to differential costs of earlier reproductive decisions in *females* [102] again arguing for a female-specific research focus.

For each of these critical reproductive traits there is marked *individual* or phenotypic variation among females within populations (Fig. 1). If we believe that hormones play a key role in determining phenotype, as endocrinologists do we have mechanisms to explain this individual variation or phenotypic plasticity? At the outset it is important to recognize that considering individual variation forces us to ask a different set of questions with regard to endocrine mechanisms [101]. A common approach in comparative endocrinology is to treat reproduction as a *qualitative*, *categorical trait*, i.e. we analyze and compare hormonal, cellular or molecular differences between groups of individuals that are either breeding (reproductively active) or not breeding. We might find that non-breeding females have baseline plasma estradiol (E2) levels whereas egg-laying females have highly elevated plasma E2 and we would conclude, probably correctly, that E2 plays an important role in regulating egg production. However, to understand hormonal mechanisms underlying *individual variation* we need to consider reproduction as a *quantitative*, *continuous trait* or complex of traits – the way that ecologists and evolutionary biologists treat most traits. Here, the appropriate question is, '*do females laying many*, *large eggs have higher plasma E2 than females laying few*, *small eggs*'? In other words, does quantitative, individual variation in plasma E2 explain individual variation in reproductive phenotype? Although we all collect hormonal data from individual animals this latter approach to data analysis and interpretation is still relatively rare [101]. Next, I will review some aspects of timing of breeding, clutch size and parental care, and ask how well we understand hormonal mechanisms regulating these traits in this context of female-specific reproduction and individual, phenotypic variation.

3. Hormonal regulation of timing of breeding

We have had a working model for control of seasonal reproduction for 40 or more years. This model suggests that day length provides reliable 'initial predictive information' for general timing of seasonal breeding, that 'supplemental factors' such as temperature, food and social cues fine-tune the actual timing of egg-laying, and that this environmental information is integrated by the hypothalamic–pituitary–gonadal (HPG) axis to regulate gonadal function [10,26,29,107]. There is substantial, experimental support for this model but, I would argue, mainly for photoperiodic control of testis maturation in male birds, and from studies of males in captivity. Male birds transferred from short-day (SD) photoperiods (8L:16D) to stimulatory long-days (LD, >13L) show an increased release of hypothalamic gonadotropin-releasing hormone (GnRH), an increase in plasma levels of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and, even in captivity, this leads to testis maturation, spermatogenesis and steroidogenesis, with elevated plasma testosterone levels [26,69,107]. In contrast, females of most non-domesticated species held in standard captive conditions (small cages) will not undergo complete ovarian development: the ovary can develop to the pre-vitellogenic phase but onset of vitellogenesis, yolk formation and egg production is very unusual in captivity [54]. Over 40 years ago Farner et al. [35] highlighted the fact that "*the accumulation of data on the ovarian cycle*, *both in the field and the laboratory*, *has been necessarily at a much slower rate* [than for testis function]" and this situation has hardly changed [6]. Unfortunately, rather than tackling the problem of regulation of ovarian function head on, perhaps solving the problem of control of timing of egg-laying – a key life-history trait – most subsequent studies side-stepped this issue by working solely on males (a bias which is also prevalent in mammalian studies [111]). It is, of course, possible to rationalize this focus on males early in the development of avian endocrinology: it could be argued that most endocrinologists in the last 40–50 years have not been trying to solve evolutionary problems *per se* and that males were the model of choice for studies investigat-

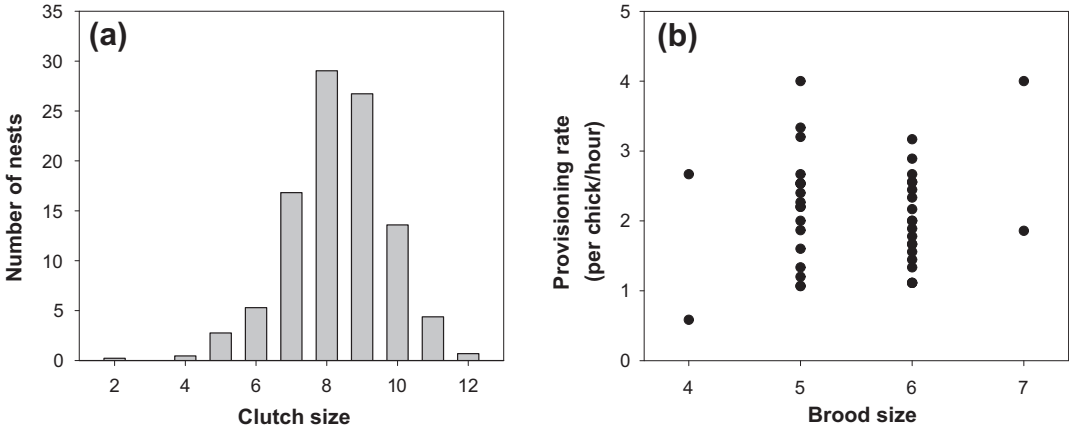

Fig. 1. Examples of phenotypic variation in reproductive traits for (a) clutch size in blue tits (data from [71]) and (b) provisioning of nestlings during parental care in European starlings. Do we have hormonal mechanisms for this variation?

ing how environmental cues turn the reproductive system "on and off" in the broader context of seasonality. It is also true that some of the earliest field endocrinology studies *did* include females and generated female-specific data on ovarian and gonadal steroid cycles which are still very valuable today [104,105]. Nevertheless, the most important point here is that females *do* show the same long-day response as males in captivity at the level of the hypothalamus and pituitary, i.e. "upstream" in the HPG axis. Female European starlings, *Sturnus vulgaris*, show more-or-less identical changes in hypothalamic GnRH to males with transfer from SD to LD, and plasma LH and FSH levels in LD-photostimulated females are actually *higher* than those in males [25]. Thus, females *do* respond to photoperiod alone at the level of the hypothalamus and pituitary, but regulation of ovarian steroidogenesis, vitellogenesis, follicle and oviduct development – that is required for onset of egg-laying – must occur downstream of the pituitary at the level of the ovary and/or liver.

To illustrate the potential value of taking a female-specific approach investigating "downstream" reproductive function I will describe some of our recent work in European starlings held under standard captive conditions. In one experiment we hand-reared chicks from 14-days of age and then exposed them to artificial photoperiods mimicking the natural day length cycle at our breeding location (54°N); two approaches *not* commonly used in most photoperiodic experiments. Although males showed robust changes in testis size under these conditions no females showed significant ovarian (yolky follicle) or oviduct development. This suggests that, a) lack of ovarian development is *not* simply due to experimental studies using the artificial paradigm of rapid transition from very short (<8L) to very long (>16L) day lengths, and b) developmental acclimation to captive conditions during rearing is *not* sufficient to lift the regulatory constraint of ovarian development in females (cf. Baptista and Petrinovich [7]). In a second experiment we investigated changes in yolk precursor production, downstream of the ovary, under SDs and LDs coupled with exogenous E2 treatment (Fig. 2). Both yolk precursors, vitellogenin and yolk-targeted VLDL (VLDLy), were basal on SD and on LD in control females not treated with E2. In LD-E2 treated females plasma VTG was elevated to levels typical of breeding birds (Fig. 2a) but plasma levels of VLDLy were typical of non-breeding birds even with LDs and exogenous E2 treatment (Fig. 2b). The major shift in lipid metabolism from synthesis of generic VLDL to VLDLy represents one of the final stages of reproductive maturation in females and we have shown elsewhere that this process is likely especially 'costly' to females [78,79] because they have only a limited ability to use VLDLy to meet their own metabolic needs [93]. These data suggest that we should expect to see the evolution of more "conservative" female-specific, regulatory mechanisms, with differential hormonal regulation of the two main yolk precursors far "downstream" in the HPG axis, e.g. at the level of the liver [diurnal control of ovulation and oviposition represent another under-studied process in free-living birds [102] and one where the HPG axis, and specifically a LH surge, is clearly critical, but I consider this perhaps as a *temporally* separate circadian process different from that initiating the *seasonal* onset of ovulation and timing of laying].

So, if we ask how well our current model for timing of breeding works for females I would argue that the answer currently is not very well at all. The strong male-bias in experimental studies has meant that we have little information on ovarian, oviduct or liver function in females for non-poultry species. Moreover, where females have been used in experimental studies they have been exposed to the same environmental cue values as males, as if they were clones of males! As Fig. 3 shows, when we consider the ecological context of timing of reproductive maturation marked sex-specific differences are apparent, and rapid gonadal maturation occurs under very different prevailing environmental cues. In starlings at 54°N, rapid gonadal maturation is initiated in late February in males but in early April in females, when day length and mean daily temperatures are ~12L and 5.1 °C, and ~15L and 7.8 °C, respectively. Current experimental paradigms largely ignore this sex-specific physiological and ecological context of timing of breeding, as well as the sex-specific evolutionary context. Males and females respond to different selection pressures and we *should* expect sex-specific regulatory mechanisms of gonadal development to have evolved. Information from day length alone, perhaps with "upstream" control from the hypothalamus, is likely sufficient to time testis maturation in males where costs of sperm production are low [44], costs of incorrect timing decisions are consequently low, but where benefits of early and prolonged fertility are high. In contrast, in females costs of egg production are relatively high [44] and there are significant fitness costs of small-scale, day-to-day variation in timing, leading to "downstream" regulation [16,100]. As Ball [5] suggested "*The ovary knows more than you think*!", and I would argue that the liver might also know more than we think!

4. Hormonal regulation of clutch size

Clutch size is probably the most well-studied avian life-history trait, and one with very strong, systematic patterns of individual variation [57] that should facilitate the search for hormonal mechanisms. In many single-brooded species there is a linear seasonal decline in clutch size with laying date over several weeks [32,64] but clutch size can also vary systematically among individuals over

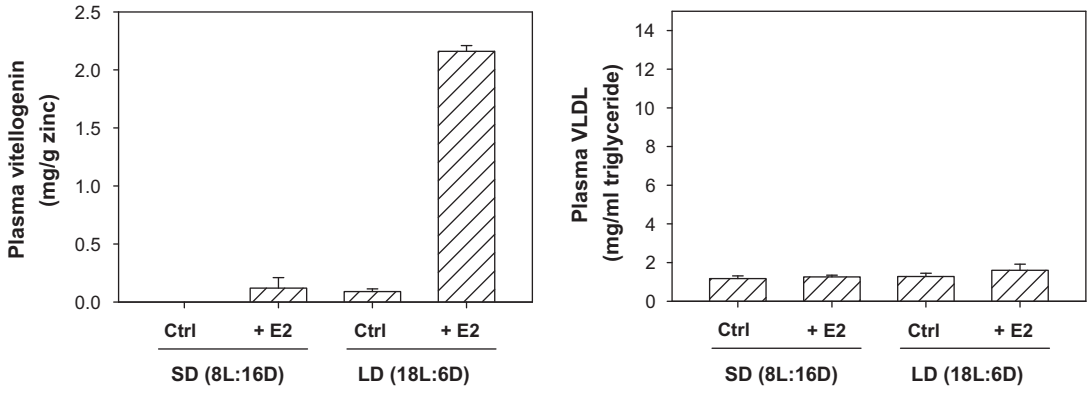

Fig. 2. Differential effects of photoperiod and exogenous 17β-estradiol treatment on the two yolk precursors, plasma vitellogenin and yolk-targeted VLDL, in female European starlings. Values are means ± S.E.

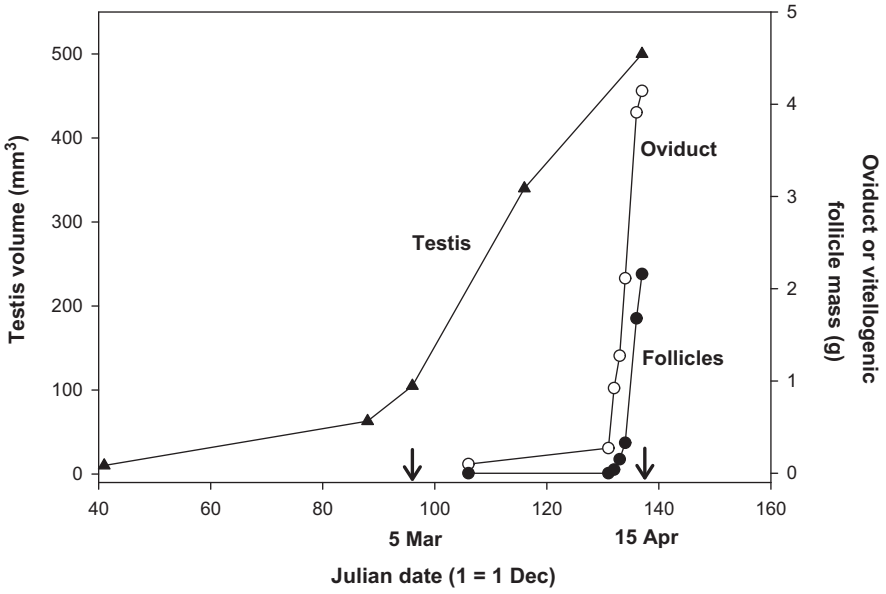

Fig. 3. Sex-specific differences in timing and rate of seasonal gonadal maturation in male (triangles) and female (circles) European starlings; data for males are from [27]; arrows indicate timing of onset of rapid gonadal growth in each sex.

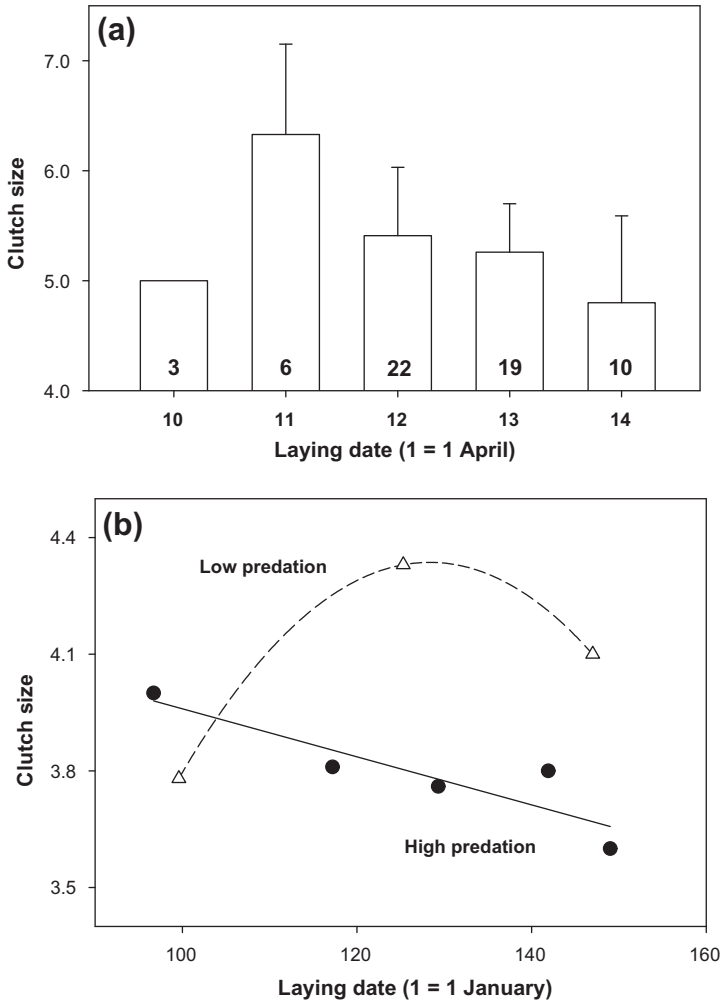

Fig. 4. Laying date-dependent phenotypic variation and plasticity in clutch size for (a) European starlings, and (b) song sparrows in a high and low predation environment (based on [90]).

just a few days in highly synchronous breeders (Fig. 4a). Multi-brooded species have a different seasonal pattern with clutch size first increasing with date then declining [20], but this can be phenotypically plastic depending on the laying environment (Fig. 4b; [90]). Any generalized endocrine mechanism must be able to explain this inter- and intra-specific variation in clutch size, including the "fine-tuning" of clutch size to the prevailing environment over time periods as short as 1–2 days, and date-independent variation or plasticity in clutch size (Fig. 4b). So do we have an endocrine mechanism to explain this individual or phenotypic variation in clutch size?

Predictive models for hormonal-regulation of clutch size determination have been around for 15–20 years but appear to have been largely ignored by endocrinologists, at least experimentally. These models were derived from studies by Meijer and colleagues in Eurasian kestrels *Falco tinnunculus* [11,64] and similar work in small songbirds by Haywood [45,46]. Both models propose the existence of an endogenous "inhibitory signal" which increases seasonally and/or during egg-laying, and is associated with development of incubation behavior, but which eventually interrupts follicle development, thus terminating ovulation and determining final clutch size (see Fig. 5). Meijer et al. [64] and Haywood [45] speculated on the physiological nature of the controlling variable underlying their models and suggested that this was a prolactin-mediated mechanism with the "inhibitory signal" involving anti-gonadal effects of prolactin (see also Meijer et al. [65] and Sockman et al. [87]). These models therefore make explicit predictions about functional relationships between prolactin and clutch size which should be easily testable in well-designed experiments. Moreover, this basic model can easily be modified to make specific predictions about *individual* variation in clutch size for a given laying date if, for example, individual females have different thresholds for the inhibitory signal that causes follicle resorption (Fig. 5a) or differences in the slope or rate of the endogenous day-length or laying dependent increase in plasma prolactin (Fig. 5b).

Despite the existence of this hormonal model I would argue that robust, experimental evidence that prolactin regulates clutch size is virtually non-existent (perhaps in part due to problems with experimental manipulation of prolactin, although use of bromocriptine, vasointestinal peptide (VIP) or immunization against VIP or PRL offer possibilities). Some work in poultry is consistent with the idea that prolactin has an inhibitory effect on ovarian function; e.g. Youngren et al. [110] demonstrated that intracranial perfusion with prolactin induces incubation behavior and is associated with a decrease in egg production in turkey hens (see also Reddy et al. [74]). However, other studies have focussed on the hypothalamus and pituitary, mainly using *in vitro* approaches [77,89,109], and it is often not clear that the demonstrated inhibitory effects of prolactin are sufficient to affect ovarian function and egg production *in vivo*. Sockman et al. [85] and Sockman and Schwabl [86] provide the only experimental data that I know of on prolactin and clutch size in a non-domesticated bird, the American kestrel *Falco sparverius*. Here, birds laying smaller clutches had higher plasma prolactin levels early in laying, consistent with an inhibitory effect of prolactin. However, experimental manipulation of plasma prolactin did not have any effect on clutch size. In a later review, Sockman et al. [87] concluded that "a role for prolactin in regulating clutch size in any species is not firmly established". I fully agree with this statement: regulation of clutch size is probably *the* most important gap in our understanding of hormonal control of avian reproduction.

5. Hormonal regulation of parental care (foraging and provisioning effort)

Continuing with the same conceptual theme, what do we know about hormonal regulation of parental care and chick provisioning? As numerous studies have shown, there is marked, largely unexplained, individual variation in chick provisioning effort (Fig. 1a). However, I do not think that we currently have a working model for physiological or hormonal determinants of this variation. It is widely assumed, based largely on behavioral ecology studies, that parental care and chick provisioning is a demanding activity – an idea that is intuitively attractive - and that parents that work "harder" benefit by rearing rear more, larger (fitter) offspring (though see Schwagmeyer and Mock [81]). However, it is also thought that high feeding rates can be costly to parents with birds becoming "fatigued" [39], or experiencing a "temporary suppression of vitality" leading to increased "risk of death by exhaustion" (Daan et al. [24]). In other words, it is widely assumed that there are *physiological* costs of reproduction associated with parental care [70,75]. Although these general ideas are entrenched in the avian ecology literature very little hormonal research has actually been conducted in the context of parental care, and the physiological basis of variation in avian foraging in general is very poorly known [62]. Numerous studies have taken an energetics approach to this problem, looking at physiological work as measured by metabolic rate or daily energy expenditure, but these have often failed to reveal the predicted simple, linear relationships with parental effort [97].

A recent study of free-living zebra finches *Taeniopygia guttata* showed that individual birds can fly *minimum* distances of between 0.4 and 19.4 km each day (mean 6.4 km) while rearing chicks [61]. If humans were to run 19 km per day for 2–3 weeks this would represent a fairly high level of exercise, and certainly one very different from running 0.4 km per day. So could chick-rearing be a problem of *exercise endocrinology*? There is an

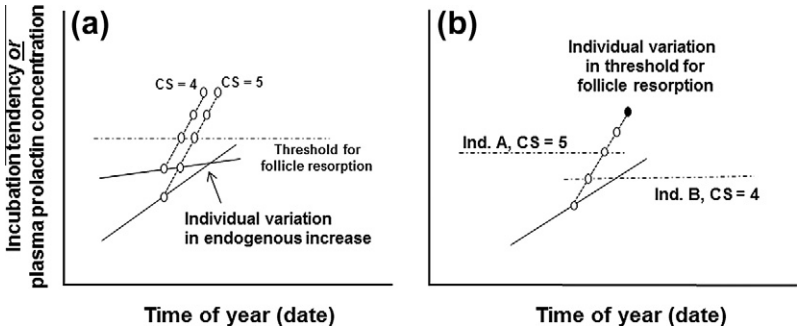

Fig. 5. Models for date-independent phenotypic variation in clutch size involving (a) individual variation in the rate of endogenous increase in incubation intensity or prolactin and (b) individual variation in the threshold for disruption of follicle development (based on Meijer et al.'s [64] original model). Open circles indicate ovulated follicles which are oviposited, closed circles indicate follicles which can potentially be reabsorbed.

abundant literature on endocrine and neuroendocrine regulation of exercise, albeit largely from human studies of kinesiology and sports medicine [13,33,55]. Concepts such as "over-reaching" and "over-training" are associated with long-term performance decrements due to intense exercise, and as any amateur athlete knows exercise can lead to "exhaustion and temporary suppression of vitality" (to use Daan's [24] words). Can this conceptual approach provide a model for physiological regulation of workload associated with chick-rearing? Numerous studies have characterized hormonal changes associated with exercise including glucocorticoids, prolactin, thyroid hormones, catecholamines, growth hormone, IGF-1, and gonadal steroids [33] so there are no shortage of candidate hormones of interest. Furthermore, parental care lends itself to experimental work since free-living birds can easily be captured, hormonally-manipulated, and released during chick-rearing without risk of nest abandonment. Finally, an intriguing suggestion in the context of female-specific costs of reproductive effort [102] is that females might be more susceptible to over-training than males [83]. This clearly represents an almost untouched area of integration of comparative endocrinology and ecology where hormonal studies could significantly contribute to our understanding of individual variation in parental care.

6. Hormonal regulation of trade-offs between traits or life-history stages

So far I have considered single traits (phenology, clutch size) or single breeding stages (chick-rearing), but I now briefly want to consider hormonal regulation of trade-offs and carry-over effects *between* different traits or different life-history stages. Two endocrine-related concepts capture the essence of evolutionary or life-history trade-offs: hormonal pleiotropy and hormonal conflict. Hormonal *pleiotropy* (a term 'borrowed' from evolutionary genetics) occurs where a single hormone has both positive and negative effects on different physiological systems or traits [36,43,53] and is a long-recognized hallmark of hormone action. Hormonal *conflict* can occur when *different* regulatory systems are required simultaneously for overlapping functions or life-history stages and the regulatory mechanisms underpinning each function generate inherent hormonal (or metabolic) incompatibilities. Both these mechanisms might operate whenever birds have to do different things at the same time. Fig. 6a presents one commonly held view of life-histories, prevalent in the literature, where different stages of the life cycle are sequentially orchestrated with minimum overlap between stages (it is argued due to high energetic costs of each stage). However, Fig. 6b presents a more accurate, though still over-simplified, view of life-histories with lots of interaction and overlap between successive stages. Using this framework I will describe some potential examples of hormonal control of trade-offs to illustrate the concepts of pleiotropy and conflict within- (egg production vs. erythropoiesis) and between life-history stages (migration vs. reproduction, and reproduction vs. molt). Again, the aim here is to highlight the value of, and opportunities in, comparative, evolutionary, field endocrinology focusing on individual variation in female-specific traits in a life-history context.

7. Hormonal pleiotropy: estrogens and "reproductive anemia"

Anemia, a transient decrease in hematocrit and plasma hemoglobin levels, appears to be a common component of reproduction in vertebrates, e.g. it is one of the most frequent complications related to pregnancy in humans [84], and also occurs routinely in birds associated with egg production [68,98]. Erythropoiesis or red blood cell production occurs in the bone marrow in birds, regulated by erythropoietin (EPO), and estrogens can inhibit hematopoiesis at several stages of cell differentiation, proliferation, and survival [12,18,72]. We have shown that reproductive anemia in egg-laying zebra finches is due to a transient estrogen-dependent inhibition of erythropoiesis, i.e. it is an example of a direct pleiotropic effect of E2 [91]. The negative pleiotropic effects of E2 occur over a relatively short time-frame limited to the period of egg production [99]. However, physiological recovery from these effects, which involves reticulocytosis or regenerative erythropoiesis, is a relatively slow, prolonged process extending through incubation and sometimes into chick-rearing [92,98]. This hormonally-mediated mechanism, arising directly from the regulatory processes underlying reproduction [42], therefore appears to operate over the longer time scales (weeks to months) at which costs of reproduction are expressed, i.e. it should be ecologically and evolutionarily significant [100].

Preliminary data suggest that E2-mediated reproductive anemia does have significant consequences for breeding success and fitness in birds. Kalmbach et al. [51] increased costs of egg production, via egg removal, in great skuas (*Stercorarius skua*) and showed that and this was associated with a greater reduction in hematocrit and red blood cell number compared with control females. Furthermore, some of these negative hematological effects persisted for up to one year, and experimental females had later egg-laying dates in the subsequent year. In contrast, experimental work on captive-breeding zebra finches suggests that the extent of reproductive anemia is independent of reproductive effort [103] but that females might instead regulate hematological status to maintain hematocrit, hemoglobin, and red blood cell number at some minimum functional level, even at a cost of reduced reproductive investment [92]. We have preliminary data for free-living European starlings showing that transient, experimental reduction of hematocrit within the physiological range (achieved using the drug

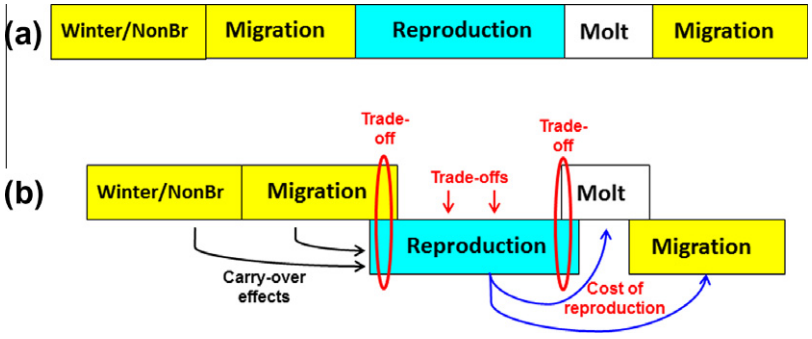

Fig. 6. Models for organization and physiological analysis of life histories: (a) sequentially orchestrated stages, in which overlap between successive life-history stages is minimized; (b) a more realistic model with overlap between successive stages integrating trade-offs, costs of reproduction, and carry-over effects among life-history stages.

phenylhydrazine) can have significant negative effects on breeding success (R. Fronstin and TDW, unpubl. data).

8. Hormonal conflict: how do birds deal with migration-reproduction overlap?

Given the relatively slow, prolonged pattern of testis maturation (see Fig. 3) it is perhaps not surprising that gonadal development can occur during migration in males [9]. Similar data for gonadal development in females of migratory species is relatively scarce. Some female surf scoters (*Melanitta perspicillata*) migrating up the Inside Passage in Alaska have elevated plasma vitellogenin levels, suggesting they have initiated rapid yolk development, while still 1200 km from the breeding grounds (John Takakawa, Matt Wilson, and TDW unpubl. data). Furthermore, intervals between arrival and laying of only 1–2 days have been reported in some migratory shorebirds [80] and passerines [94], compared to minimum durations of rapid yolk development of 4–5 days, suggesting that overlap between migration and reproductive development might not be uncommon in female birds (as with other taxa [21]). This could represent an example of 'hormonal conflict' if the different regulatory systems underlying migration and reproduction generate inherent hormonal incompatibilities.

One clear example of overlap between migration and egg formation which appears to involve hormonal conflict occurs in the *Eudyptes* or crested penguins [22]. Crested penguins have a unique pattern of reproductive investment, with extreme intra-clutch egg size dimorphism where the first-laid A-egg is 55–85% of the size of the second-laid B-egg. In macaroni penguins (*Eudyptes chrysolophus*) small A-eggs are viable but >85% of A-eggs are lost within 1 day of the start of incubation and <0.4% produce chicks [96]. Thus, most *Eudyptes* penguins lay a 2-egg clutch but only ever rear one chick which appears to represent a maladaptive reproductive strategy. Can a *hormonal* perspective help resolve this problem? Based on detailed work on *female* reproductive physiology [3,41] timing of ovarian development relative to migration and arrival at the breeding ground can be accurately modelled in penguins. Egg production takes 20–25 days in *Eudyptes* spp. with the smaller A-egg developing 4 days earlier than the larger B-egg. However the interval between arrival and egg-laying varies from only 4–15 days so most bird are forming yolks at sea and, in some cases, the majority of yolk formation is occurring at sea [22] while birds are still migrating back to the breeding colonies (Bost et al., 2009). Female macaroni penguins with shorter pre-laying intervals, i.e. with greater migratory overlap, lay more dimorphic eggs and they are also less "*reproductively ready*" at arrival, with lower plasma yolk precursor levels [22]. Thus, the pattern of reproductive investment in *Eudyptes* appears to arise from a hormonally-mediated conflict between migration and reproduction. Moreover, the hormonal or reproductive "signature" of this conflict is observed downstream in the HPG axis (at the level of the ovary and liver) not upstream (e.g. plasma LH is elevated on arrival in adult macaroni penguins, and immature non-breeders can elevate plasma LH to high levels with a GnRH challenge [95]).

A second example of a downstream, hormonally-mediated mechanism linking migration-reproduction overlap, reproductive readiness and breeding decisions which might involve hormonal conflict occurs in female black-browed albatrosses (*Thalassarche melanophris*; Crossin and Williams unpubl. data). When female black-browed albatrosses return to the breeding colonies they make one of three reproductive 'decisions': they can defer breeding, they can attempt to breed but fail, or they can breed successfully. Most adult females return to the breeding colonies, and it is parsimonious to suppose that day-length drives the general timing of this return, and that the hypothalamus is "switched on" in all birds even though they subsequently make different reproductive decisions. In addition, as with macaroni penguins, plasma LH is already elevated on arrival in black-browed albatross with no differences between the sexes [47]. So what endocrine mechanisms regulate individual breeding decisions at or after arrival? Analysis of plasma hormone profiles in females at arrival clearly show that all females are *not* equally "reproductively ready" in terms of downstream components of the HPG axis during this migration-reproduction transition. Deferring females have low progesterone (P4), low plasma testosterone (T), and low plasma vitellogenin (VTG) levels on arrival, suggesting that the decision to defer is made prior to arrival perhaps due to events or environmental conditions encountered during winter or migration at sea, i.e. a potential carry-over effect. As would be predicted successful females have high plasma P4 and high plasma VTG but low plasma T, presumably due to conversion of T to E2 to support vitellogenesis. However, failed breeders are especially interesting in that they have high plasma P4 and T, but low VTG which suggests a possible disruption of aromatisation and/or the E2-mediated vitellogenesis pathway. Rather amazingly, plasma VTG at arrival not only predicts breeding outcome but it also predicts time to breeding failure among failed breeders. Here the physiologically and hormonally-relevant differences between females with different reproductive readiness, making different reproductive decisions, therefore appears to be occurring *downstream* at the level of the ovary and liver, via E2-dependent regulation of vitellogenesis.

9. Hormonal conflict: how do birds deal with breeding-molt overlap?

As a second example of hormonal conflict I want to consider breeding-molt overlap. Breeding-molt overlap is common in a wide range of avian species; most often molt is initiated during chick-rearing in the later stages of breeding [37,48,88] but in several species molt can be initiated during incubation or even during egg-laying [8,49]. This is a classic example of a life-history trade-off so, taking a hormonal approach, which hormones are potentially in 'conflict' during breeding-molt overlap, and how is this conflict resolved? One potential candidate hormone for 'conflict' is prolactin. Early studies established a role for prolactin in the regulation of parental behavior in birds [34,59] and this idea continues to dominate studies of parental care [2]. This suggests that plasma prolactin levels should be elevated to support foraging and chick-provisioning [4]. In contrast, Dawson [28] and Dawson et al. [30] provide evidence that molt is associated with decreasing plasma prolactin, and is inhibited when plasma prolactin is increasing or high. So, do birds with breeding-molt overlap have high PRL (favouring parental care) or low PRL (favouring molt)? A second candidate hormone for conflict is corticosterone (Cort). Cort is thought to play a role in regulating energy balance and food acquisition, via increased locomotor activity [14] or foraging behavior [73], and this would predict that breeding birds should elevate corticosterone to meet the energetic and behavioral demands of raising offspring [60]. In contrast, recent work by DesRochers et al. [31] and Lattin et al. [58] suggest that elevated plasma Cort levels during molt are associated with decreased feather quality, which would explain why Cort levels are basal during molt compared with other stages of the annual cycle [76]. So, do birds with breeding-molt overlap have high Cort (favouring parental care) or low Cort (favouring molt)? In this context I think it is important to consider plasma Cort in free-living birds as a continuously distributed, phenotypic trait rather than a bi-modally distributed trait with "baseline" (low) and "stress-induced" (high) levels. These concepts really represent an artificial construct that perhaps only applies to the experimental paradigm we use to

measure Cort reactivity (the standardized handling approach) and which might not be that relevant (or helpful) from the perspective of phenotypic variation in Cort and fitness. Rather, over a large range of values, continuous, phenotypic variation in plasma Cort should reflect this hormone's role as a "metabolic regulator" adjusting to meet the varying physiological demands of predictable events such as reproduction, molt and migration.

We have investigated the role of prolactin and corticosterone in molt-breeding overlap in the giant petrel (*Macronectes* spp.), a species which begins molting during incubation or early chick-rearing [23]. Plasma prolactin levels were elevated during the immediate post-hatching period consistent with a role for PRL in parental care. However, in female Southern giant petrels (*Macronectes giganteus*) and both sexes of Northern giant petrels (*Macronectes halli*), plasma prolactin levels decreased coincident with the initiation of molt, consistent with Dawson [28]. Baseline plasma Cort was also low throughout chick-rearing and initiation of molt. Thus, these hormone data are consistent with the idea that during molt-breeding overlap giant petrels prioritise a regulatory system underpinning molt (low prolactin, low Cort), perhaps in order to maintain feather quality. However, this species also clearly manages to maintain parental care, by provisioning their young left at the nest, despite having low plasma prolactin levels. In contrast, male Southern giant petrels began molting early in incubation when prolactin was increasing and had not yet started to decrease (cf. Dawson [28]). We speculate that molt in male Southern giant petrels might be regulated by some alternate physiological pathway (e.g. involving thyroid hormones) so as to avoid the negative effects of low prolactin on incubation behavior, prioritising parental care, when molt starts very early in reproduction. Either way these comparative data challenge our general ideas on the role of prolactin and Cort in hormonal regulation of parental care, nutritional stress, foraging and molt and the *interaction* of these two functions. It could be argued that giant petrels have a unique ecological context which does not help us understand the hormonal regulation of molt and breeding in general. However, I would argue that one of the strengths of comparative endocrinology is that we can utilize the ecological and evolutionary context of free-living animals to test, validate, and extend our *general* mechanisms to explain intraspecific diversity, and individual, phenotypic variation.

10. Conclusion: there are big opportunities for *endocrinologists* in evolutionary endocrinology

Uncovering the details of the endocrine system(s) that regulate phenotypic variation in those reproductive life-history traits that contribute most to individual variation in fitness: timing of breeding, clutch size, parental care, represents both a great opportunity and a worthy, if somewhat overdue, goal for the future. Endocrinologists know that hormones are key regulators of phenotype so this is a task for comparative endocrinologists, albeit working in an evolutionary or life-history framework. Extending this "opportunity" to elucidate endocrine regulation of *interactions* between different traits or different life-history stages will be much more challenging; a major impediment at present is our lack of detailed knowledge of endocrine regulation of each life-history stage, even when considered in isolation. For example, our ability to address interactions between parental care and molt are hampered by uncertainty as to the 'parental' hormone in birds [1,102] and the key hormones regulating molt [28,56]. Nevertheless, two endocrine concepts: hormonal pleiotropy and hormonal conflict capture the essence of evolutionary trade-offs at a mechanistic level, so endocrinologists can play an important role here utilizing the great strength of comparative (field) endocrinology and the power of evolutionary and ecological diversity. Finally, in pursuing these opportunities we should not lose sight of the peripheral endocrine system, or of sex-specific regulatory systems that have likely evolved due to very different selection pressures acting on females and males.

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