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Review

Hormone-mediated suites as adaptations and evolutionary constraints

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Hormones mediate the expression of suites of correlated traits and hence may act both to facilitate and constrain adaptive evolution. Selection on one trait within a hormone-mediated suite may, for example, lead to a change in the strength of the hormone signal, causing either beneficial or detrimental changes in correlated traits. Theory and empirical methods for studying correlated trait evolution have been developed by the field of evolutionary quantitative genetics, and here we suggest that their application to the study of hormone-mediated suites may prove fruitful. We present hypotheses for how selection shapes the evolution of hormone-mediated suites and argue that correlational selection, which arises when traits interact in their effects on fitness, may act to alter or conserve the composition of hormone-mediated suites. Next, we advocate using quantitative genetic methods to assess natural covariation among hormone-mediated traits and to measure the strength of natural selection acting on them. Finally, we present illustrative examples from our own work on the evolution of testosterone-mediated suites in male and female dark-eyed juncos. We conclude that future work on hormone-mediated suites, if motivated by quantitative genetic theory, may provide important insights into their dual roles as adaptations and evolutionary constraints.

Keywords: hormones; testosterone; adaptation; constraint; correlational selection; phenotypic integration

1. INTRODUCTION

Natural selection occurs when some traits are better suited to the environment than others, but the rate at which a trait evolves in response to selection often depends upon interactions with other traits within the total phenotype. When traits under natural selection are completely independent of one another, evolutionary responses may occur freely, and a given trait may quickly adapt to its environment. However, when traits depend on common mechanisms for their expression, they may evolve as a unit, potentially constraining response to natural selection (Maynard Smith *et al.* 1985; Arnold 1992; Schluter 1996). When correlations among traits are very strong, evolution of individual traits may be completely constrained. It is probable that almost all real cases lie somewhere between these two extremes, and that most adaptive evolution depends on interplay between external selective forces and internal constraints.

Hormones have been suggested to play roles in both sides of this interaction. First, they may mediate suites of traits that together create a better fit to an organism's environment and thus may be seen as adaptations. Alternatively, by linking the expression of multiple traits, hormones may constrain their independent evolution. In this paper, we explore the evolution of hormone-mediated suites, focusing on their dual roles as adaptations and constraints. First, we briefly review the quantitative genetic theory of the evolution of correlated characters and apply this framework to the evolution of hormone-mediated suites, which we argue are analogous to genetic correlations. Building from this framework, we next examine selective mechanisms that may affect the evolution of hormone-mediated suites. We suggest that the origin and maintenance of hormone-mediated suites may often be favoured by correlational selection, and that changes in the strength and direction of correlational selection should lead to changes in these suites. We then propose research directions that should help illuminate the microevolutionary processes that affect (and are affected by) hormone-mediated suites. Finally, we present examples from our own work on dark-eyed juncos that explore the potential for testosterone-mediated suites to act as both adaptations and evolutionary constraints.

2. GENETIC CORRELATIONS AND HORMONE-MEDIATED SUITES

Darwin (1859) noted the generality of 'correlations of growth', speculating that when changes in one character are 'accumulated through natural selection, other parts become modified' (p. 143). Lande (1979) and Lande & Arnold (1983) incorporated this observation into a general mathematical model of the

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evolution of correlated traits in response to natural selection. Lande's 'multivariate breeders' equation, $\Delta\bar{\boldsymbol{z}} = \boldsymbol{G}\boldsymbol{\beta}$, describes how the evolutionary response of an interrelated set of traits ($\Delta\bar{\boldsymbol{z}}$, a vector) to selection (specifically, directional selection, represented by $\boldsymbol{\beta}$, a vector of 'selection gradients') is influenced both by additive genetic variance in a particular trait (diagonal elements of the matrix $\boldsymbol{G}$) and by additive genetic covariance among traits (off-diagonal elements of $\boldsymbol{G}$). Additive genetic variance (and its standardized form, heritability) represents the amount of variation in a trait that is heritable. Additive genetic covariance (and its standardized form, the genetic correlation) represents the degree to which traits are co-inherited due to pleiotropy, which is the common effect of loci on multiple traits, and/or to linkage disequilibrium, which is the physical or statistical linkage of loci affecting one trait to loci affecting a second trait (Lande 1980*a*).

Genetic correlations cause multiple traits to respond to selection as a unit, and depending on the direction of natural selection, they may accelerate or impede response to selection. The accelerating effect has received much attention in the study of sexual selection, where genetic correlations between the sexes in trait and preference can lead to the evolution of extreme ornaments (Lande 1981). By contrast, genetic correlations among traits within a sex are more often seen as measures of genetic constraint on phenotypic evolution (Cheverud 1984; Maynard Smith *et al.* 1985; Arnold 1992; Blows & Hoffmann 2005). The effect of directional selection on a suite of correlated traits depends both on the pattern of genetic covariances ($\boldsymbol{G}$) and the direction of the vector of selection gradients $\boldsymbol{\beta}$. Simplistically, response to selection should be most rapid when selection acts on combinations of traits that, collectively, have the most genetic variance (Schluter 1996; Blows & Hoffmann 2005). When we consider a two-trait example, a response to selection should be most rapid when the direction of selection is aligned with the major axis of the correlation between two traits (figure 1). With two positively correlated traits, this occurs when selection favours a simultaneous increase in both traits. Response to antagonistic selection, which aligns with the minor axis of the correlation, should be more difficult. In other words, a positive genetic correlation may prevent a response to selection for a decrease in one trait and an increase in the other (figure 1).

From another perspective, genetic correlations may be seen as adaptations as well as evolutionary constraints (Merilä & Björklund 2004). Patterns of covariation may arise because selection favours the integration of traits performing a common function (Olson & Miller 1958; Cheverud 1982). Theory predicts that selection, particularly a form known as correlational selection, should be able to change the magnitude of genetic covariances over time (Lande 1980*a*; Lande & Arnold 1983; Phillips & Arnold 1989; Rice 2000; Sinervo & Svensson 2002; Jones *et al.* 2003; Phillips & McGuigan 2006). Correlational selection describes situations in which the fitness effects of two traits are interactive (or epistatic) rather than additive; that is, the selective advantage of one trait depends on the value of another. When correlational selection favours a certain combination of traits, the genetic covariance between them is predicted to increase over time (Lande 1980*a*; Phillips & Arnold 1989; Phillips & McGuigan 2006). Indeed, correlational selection has been shown to coincide with existing genetic correlations in a number of cases (Brodie 1989, 1992; Conner & Via 1993; Morgan & Conner 2001; McGlothlin *et al.* 2005, but see Blows *et al.* 2004). In these studies, selection favoured combinations of traits that were known to be genetically correlated, as one would predict if past selection accounted for the present existence of the genetic correlations.

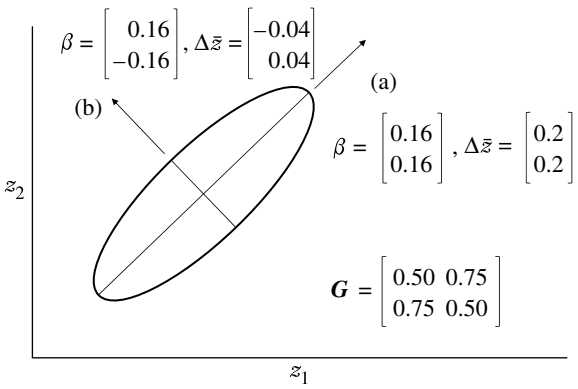

Figure 1. Schematic of the effect of genetic covariance on selection response. The magnitudes of the elements of $\boldsymbol{G}$ were chosen arbitrarily. The diagonal elements of $\boldsymbol{G}$ represent additive genetic variance for each trait, z_1 and z_2, and the off-diagonal element represents a positive genetic covariance between the two traits. Directional selection is represented by the vector $\boldsymbol{\beta}$, and the magnitude of the elements is the median from studies of natural populations (Kingsolver *et al.* 2001). Response to selection acting in the same direction on both traits, that is, on the major axis of the correlation (a) is facilitated by genetic covariance, and the selection response ($\Delta\bar{\boldsymbol{z}}$) is positive for both traits. Response to selection acting in opposite directions, that is, on the minor axis (b) is constrained, as shown by the small values in $\Delta\bar{\boldsymbol{z}}$.

Because hormones coordinate the expression of multiple aspects of physiology, morphology and behaviour, they are often considered physiological analogues of genes with pleiotropic effects (Finch & Rose 1995; Ketterson & Nolan 1999). Hormones often serve as a link between an organism's environment and the expression of an appropriate phenotype. The physiological details of this linkage are usually complex; for simplicity, we refer to a set of interconnections among environment, hormones and phenotypic expression as a hormonal pathway. Many aspects of such pathways are likely to vary among individuals, generating phenotypic variation (Ball & Balthazart 2008; Hau 2007). For example, individuals may differ in the rate of hormone synthesis, release and degradation, leading to variation in circulating hormone levels. There is ample room for individual variation in how a hormonal pathway responds to environmental stimuli, as well as how hormones interact with each other or with target tissues to cause phenotypic effects. In some cases, this variation has been shown to have a genetic basis (Evans *et al.* 2006; Kempenaers *et al.* 2008).

Like genetic correlations, hormonal correlations arising from common mechanistic mediation of multiple aspects of the phenotype may be seen both

as adaptive products of past selection and as potential constraints on future evolution. One case where this dual nature is apparent is in the role of hormones as physiological mediators of life-history trade-offs (Ketterson & Nolan 1992; Sinervo & Svensson 1998; Zera & Harshman 2001; Ricklefs & Wikelski 2002). Hormonal mediation may allow an organism to optimally allocate limited time or energy among competing functions such as growth, reproduction or immune function. However, this hormonal mediation may also impede a population's ability to respond to novel selection pressures encountered in a new environment, particularly if selection favours new relationships among hormone-mediated traits.

Available evidence suggests that hormonal mediation is not likely to lead to absolute constraints on adaptive evolution; that is, hormone-mediated suites can and do evolve (Adkins-Regan 2008; Hau 2007). Comparative studies clearly show that some elements of hormonal pathways differ among closely related species, while other elements remain conserved even across distantly related taxa (Adkins-Regan 2005). Furthermore, hormonal traits have been shown to respond to both direct and indirect artificial selection (e.g. Carere *et al.* 2003; Øverli *et al.* 2005; Evans *et al.* 2006).

3. HOW DOES SELECTION AFFECT HORMONE-MEDIATED SUITES?

Despite the mounting evidence that hormonal pathways evolve, few studies have examined the microevolutionary processes at work in natural populations. In this section, we emphasize the utility of the quantitative genetic concept of phenotypic selection (Lande & Arnold 1983) for pursuing such studies. Because natural selection acts most directly on phenotypes that interact with the environment, we focus on selection acting on the traits that make up hormone-mediated suites—the phenotypes that directly interact with the biotic and abiotic environment—rather than on the components of the hormonal pathway itself. More concretely, our analysis addresses how selection acts on correlated suites of behavioural and morphological traits mediated by hormones, with potential evolutionary effects on the physiological steps relating hormone synthesis, hormone release and hormonal impact on cellular metabolism.

The effects of selection on hormone-mediated suites, and the extent to which hormonal regulation acts to enhance or impede evolution, are likely to depend on the mode of selection (the shape of the relationship between phenotype and fitness) as well its strength and consistency (whether it fluctuates or continues in the same direction for many generations). The most common mode of selection in natural populations, and the process accounting for most adaptive evolutionary change, is thought to be directional selection (Kingsolver *et al.* 2001; Rieseberg *et al.* 2002; Estes & Arnold 2007).

As discussed in §2, genetic correlations can drastically alter the response to directional selection (figure 1). Similarly, mediation by a common hormone may facilitate a simultaneous increase or decrease in two traits in response to selection that aligns with the major axis of the correlation. This type of selection may lead to an evolutionary increase in the circulating hormone signal or a coordinated change in receptor expression across multiple tissues. One hypothetical example of such a case is selection for greater repertoire size and song frequency in a songbird. These traits might require more highly developed vocal control nuclei and greater development of the syringeal musculature. Both of these targets are androgen sensitive (Konishi 1985). If selection favoured an increase in both traits, then both might respond readily, and testosterone levels or receptor expression may evolve as a consequence. Many artificial selection experiments that have been conducted on hormone-mediated suites resemble natural selection on the major axis. For example, many studies have selected on a correlated suite of traits such as 'personality' (Adkins-Regan 2005). This sort of selection may be common in the wild as well. For example, selection for simultaneous increases in testosterone-mediated traits may be favoured when sexual selection on males is strong.

At the other extreme, selection along the minor axis to uncouple correlations might be constrained by hormonal mediation, at least in the short term. Returning to our previous example, if selection were to favour both an increase in repertoire size and a decrease in song frequency, the response may be slower owing to their common dependence on androgens. This type of evolutionary constraint is likely to be most important when selection pressures fluctuate over time. For example, if selection acting on song frequency oscillated in direction from year to year, correlations with other testosterone-mediated traits should constrain its response to selection, stabilizing the hormone-mediated suite.

However, we predict that when selection to uncouple correlations is strong and consistent, as might be expected when a population colonizes a new environment, the power of hormonal mediation to act as a constraint may be overcome. This may occur because correlations among traits can themselves evolve. One major force that may alter genetic correlations is correlational selection, which, as described in §2, acts when the effect of one trait on fitness depends on its co-expression with another trait (Lande & Arnold 1983). Correlational selection has rarely been detected in natural populations, probably because few studies have set out to measure it (and due to the large sample size required to do so; Kingsolver *et al.* 2001). Despite the paucity of measurements, Kingsolver *et al.* (2001) suggest that it may be the most common mode of selection. The classic example of correlational selection comes from an experiment on garter snakes (*Thamnophis ordinoides*) by Brodie (1992), who showed that spotted snakes were more likely to survive if they performed evasive behaviour, whereas striped snakes survived better if they did not perform the behaviour.

Correlational selection can cause evolutionary change in the genetic covariance between traits. The change in the $\boldsymbol{G}$ matrix in one generation due to selection may be predicted using the equation $\Delta\boldsymbol{G} = \boldsymbol{G}(\boldsymbol{\gamma} - \boldsymbol{\beta}\boldsymbol{\beta}^{\mathrm{T}})\boldsymbol{G}$, where $\boldsymbol{\gamma}$ is a matrix representing nonlinear selection (quadratic—stabilizing and disruptive—selection is on

the diagonal and correlational selection is on the off-diagonal) and $\boldsymbol{\beta}\boldsymbol{\beta}^{\mathrm{T}}$ is the squared effect of directional selection (T denotes transposition, which in this case simply converts a column vector to a row vector; Phillips & Arnold 1989, following Lande 1980*a*). This equation is mathematically complicated, but certain generalizations can be made. Acting alone, directional selection tends to degrade genetic variances and covariances. Although its net effect depends upon interplay with both directional and quadratic selection, correlational selection has the most direct, and potentially the strongest, effect on genetic covariance. When correlational selection is positive, genetic covariance tends to increase, whereas negative correlational selection causes covariance to decrease (or become more negative). Under certain assumptions and adding estimates of mutation and recombination, these conclusions can be extended to across-generation changes (Jones *et al.* 2003; Phillips & McGuigan 2006).

Applying this approach to hormone-mediated suites, we predict that correlational selection should also be important in the evolution of the composition of such suites. When correlational selection acts, it inherently generates linkage disequilibrium—statistical association between loci—by differential survival or reproduction of individuals with the 'right' combinations of alleles. However, it also favours alleles that generate pleiotropy between the traits under selection, which provides more stability in the face of recombination. To the extent that these pleiotropic variants are generated by a hormonal pathway, the association of traits within a hormone-mediated suite may be strengthened or weakened by correlational selection. Stated another way, correlational selection arising from the effects of coordinated co-expression of traits may have effects on the co-sensitivity of target tissues to a hormonal signal by favouring or disfavouring coordinated expression of traits.

Rice (2000) presents a mathematical and visual model that shows how correlational selection can alter developmental relationships, increasing the integration of the phenotype by associating traits with common 'underlying factors'. If those factors are hormones (or other aspects of a hormonal pathway), and correlational selection acts consistently enough, selection may lead to the addition of traits to hormone-mediated suites. Owing to the complexity of hormonal pathways, evolutionary change may occur at many different steps along the hormonal pathway (Nijhout 2003). The most probable type of pleiotropic mutation may be one that affects the expression of hormone receptors. Hormones have no effect on a tissue unless it expresses the appropriate receptor, and the expression of a receptor in a novel location or with novel timing could allow a trait to be co-opted into a hormone-mediated suite. Co-option of existing physiological mechanisms is probably common because it requires fewer evolutionary steps than building a new pathway de novo. Nijhout (2003) suggests such a scenario for the evolution of horn polymorphism in *Onthophagus* beetles.

Other forces, such as directional selection, recombination and mutation, may break up correlations among traits. Although hormonal mediation (and pleiotropy in general) may act as a buffer against such change, especially in the short term, the pattern of covariance among traits may change significantly if these forces act consistently over a long period of time. Correlational selection, as the multivariate analogue of stabilizing selection, is expected to provide stability to groups of correlated traits when it is aligned with $\boldsymbol{G}$ (Blows & Brooks 2003; Estes & Arnold 2007).

By analogy, we expect that once hormone-mediated suites have evolved, owing perhaps to correlational selection, they should also be reinforced over the long term by correlational selection, but that traits may be lost or gained when the selective landscape changes;

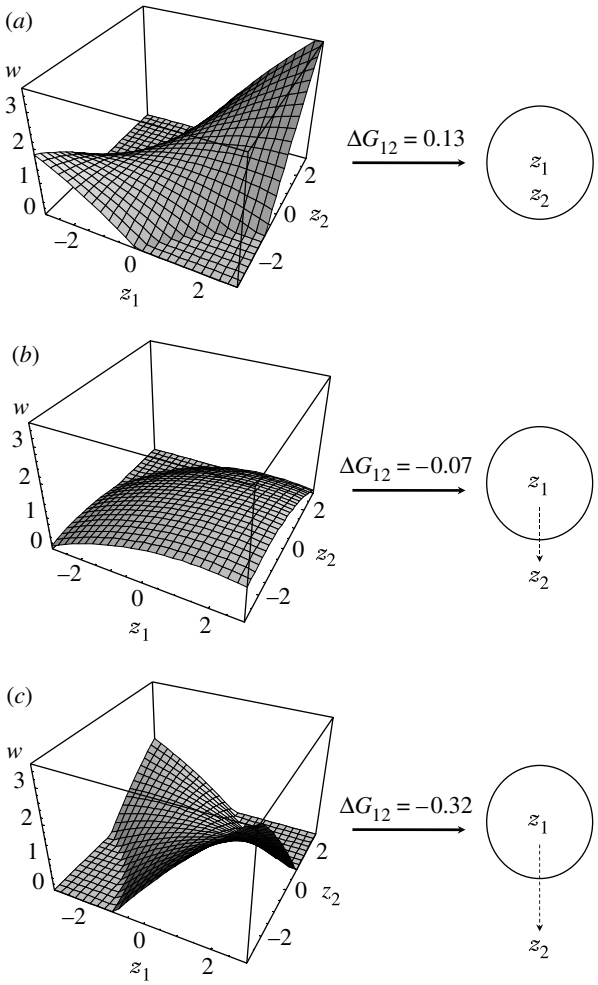

Figure 2. Hypothetical example of the effect of correlational selection on hormone-mediated trait suites. The plots on the left are individual fitness surfaces, with two traits, z_1 and z_2, on the horizontal axes and relative fitness, w, on the vertical axis (Brodie *et al.* 1995). In (*a*), the two traits are regulated by a common hormone, as represented by the circle on the right. As in figure 1, each has a genetic variance $G=0.5$, and the genetic covariance is $G_{12}=0.75$. The fitness surface shows natural selection on the two traits. Both traits are under moderate directional selection, $\beta=0.16$, and stabilizing selection, $\gamma=0.1$ (medians from Kingsolver *et al.* 2001), and are affected by relatively strong correlational selection, $\gamma_{12}=0.3$. Using the equation $\Delta\boldsymbol{G}=\boldsymbol{G}(\boldsymbol{\gamma}-\boldsymbol{\beta}\boldsymbol{\beta}^{\mathrm{T}})$, this selective regime is predicted to maintain the correlation between the traits, and hence, their common hormonal basis. In (*b*), the direction of selection on z_2 is reversed ($\beta=-0.16$), and there is no correlational selection. Within one generation, a decrease in the genetic correlation is predicted. Over several generations, z_2 may become disassociated from hormonal regulation. In (*c*), negative correlational selection ($\gamma_{12}=-0.3$) also occurs, accelerating the dissociation of z_2.

figure 2 illustrates this idea. (The graphs in figure 2 are fitness surfaces, which are three-dimensional representations of selection in a population (Brodie *et al.* 1995). Trait combinations with high fitness (*w*, plotted on the vertical axis) are favoured by selection.) In figure 2*a*, individuals that have high values of two traits have higher fitness and the traits' effects on fitness interact; that is, they are under both directional and correlational selection, creating a rising fitness ridge. Proximately, the correlation between the traits is generated by a common hormonal mechanism, while ultimately this hormonal mechanism is maintained by correlational selection, which acts to strongly disfavour variants that lead to a loss of hormonal regulation of one of the traits (e.g. by turning off the expression of the receptor in a specific tissue).

Figure 2*b*,*c* shows how traits may be decoupled from a hormone-mediated suite. Imagine the population has colonized a new environment where one of the traits is now disfavoured. Correlational selection is also reduced, because the two traits are no longer favourable when expressed together. In this case, the genetic covariance between the traits is predicted to decrease. Mutations that decouple one of the traits from hormonal regulation may now invade, and the composition of the hormonal suite may change in a number of generations. Epistatic fitness effects may also arise in the new environment, causing negative correlational selection, which would further accelerate the loss of a trait from hormonal mediation ('deintegration'; *sensu* Rice 2000). Such a change in the fitness surface may be responsible for a disassociation of traits from hormone-mediated suites, such as parental care behaviour in chestnut-collared longspurs (*Calcarius ornatus*: Lynn *et al.* 2002).

4. APPLICATION OF QUANTITATIVE GENETIC METHODS TO HORMONE STUDIES

To date, many of the studies examining potential effects of hormones on adaptation and constraint have used 'phenotypic engineering' (e.g. Ketterson & Nolan 1999; De Ridder *et al.* 2000; Reed *et al.* 2006). These studies, which typically examine the effects of experimentally enhanced hormone levels on behaviour or components of fitness, allow inference of physiological mechanisms and experimental tests of adaptive hypotheses. However, they provide only limited insight into the evolutionary processes that shape hormone-mediated suites. Phenotypic engineering should be accompanied by studies that are motivated by quantitative genetic theory if we are to understand the evolution of hormone-mediated suites more fully.

One of the first steps is to quantify patterns of natural (co)variation within the population of interest. Phenotypic engineering studies may show that multiple traits are affected by a hormone, but this does not necessarily translate to natural covariation. Ideally, we would be able to estimate $\boldsymbol{G}$ in order to predict the response of hormone-mediated traits to selection. While this is often quite difficult (though not impossible) to achieve in the field, estimates of the patterns of phenotypic variances and covariances ($\boldsymbol{P}$) may provide reasonable estimates of $\boldsymbol{G}$. Even measuring $\boldsymbol{P}$ poses difficulties, however, because both behaviour and hormones are notoriously variable within individuals. Using multiple measurements and standardized behavioural and physiological assays (such as simulated territorial intrusions, stress series and hormonal challenges) may alleviate this problem to some degree (Wingfield *et al.* 1997; Jawor *et al.* 2006; Goymann *et al.* 2007). Promisingly, studies measuring individual (co)variation for multiple behavioural and hormonal traits are becoming more common (e.g. Bell 2007; Kempenaers *et al.* 2008; Pinxten *et al.* 2007; Williams 2008).

In §3, we speculated as to how selection may act on hormone-mediated suites. There have been fewer measurements of selection on physiology, behaviour and life history than selection on morphology, presumably owing to the difficulty of measuring such traits (Kingsolver *et al.* 2001). In order to measure selection on hormone-mediated suites of traits, one needs a large sample of multiple traits and relative fitness measured on the same individuals. Because such hormone-mediated traits are often behavioural or life-history traits, the sampling effort required may be substantial. In addition, larger sample sizes are required to measure correlational selection than directional selection.

Measurements of selection on individual variation in hormones (as opposed to the entire suite of hormone-mediated traits) may also provide insight. Blows & Brooks (2003) argue that correlational selection may be understood by rotation of $\boldsymbol{\gamma}$ to generate measurements of quadratic (stabilizing or disruptive) selection on linear combinations of traits. This suggests that a measurement of quadratic selection on physiological measurements such as hormone levels, though not providing information about which traits contribute to fitness differences, may indicate the action of correlational selection on the multivariate suite. In one nice example, corticosterone levels were found to be under stabilizing selection in cliff swallows (*Petrochelidon pyrrhonota*; Brown *et al.* 2005).

In addition to studies of selection in natural populations, artificial selection studies can provide experimental confirmation of the malleability or rigidity of constraints (Conner 2003; Fuller *et al.* 2005). An artificial selection experiment in a butterfly (*Bicyclus anynana*) has demonstrated a response to selection despite a very strong genetic correlation between morphological characters (Frankino *et al.* 2005). Selection was applied in both directions along the minor axis of the correlation (where evolutionary response should be most constrained), creating both large/small and small/large lines. The means of both phenotypes evolved independently, but the genetic correlation remained; that is, the intercept but not the slope of the relationship was altered. To our knowledge, no such studies have been attempted on hormone-mediated traits. This might be accomplished by applying simultaneous selection for decreased song rate and increased feeding rate (and vice versa) in a male songbird. Another important avenue of research would be to apply artificial correlational selection to genetically correlated traits in an attempt to strengthen or weaken the correlation. Such studies, which to our knowledge have not yet been carried out for any traits, hormone-mediated or not, should provide insight into the persistence of constraints.

Another approach that may have a hormonal parallel is the application of artificial selection in one sex and the measurement of evolutionary response in the other. Genetic correlations between male and female traits may cause coevolution of the sexes in response to selection on one sex (Lande 1980*b*; but see Reeve & Fairbairn 2001). When the trait in question trades off with another trait related to fitness, as is so often the case for hormone-mediated traits, such a constraint may be difficult to surmount. In a dioecious plant (*Silene latifolia*), Delph *et al.* (2004) showed that selection for larger flower size in females caused a decrease in flower number in males and vice versa. These two traits are negatively genetically correlated and represent a life-history trade-off, with flower size more important for female fitness and flower number more important for male fitness (Steven *et al.* 2007). Because hormones commonly play a role in sexual differentiation, similar studies conducted on hormone-mediated traits may reveal strong evolutionary constraints.

5. ADAPTATION AND CONSTRAINT IN DARK-EYED JUNCOS

Using a phenotypic engineering approach, Ketterson, Nolan and colleagues asked whether suites of hormone-mediated traits could represent both an adaptive outcome of past selection and a constraint to future evolution. In an early series of studies, they manipulated plasma hormone levels, identified a suite of testosterone-sensitive traits, and assessed their interactive relationship to fitness (reviewed in Ketterson & Nolan 1992, 1999; Reed *et al.* 2006). Their subjects were free-living males of a songbird species, the dark-eyed junco (*Junco hyemalis*). Half the males in the population were treated with testosterone implants (T-males) at a dose that mimicked prolonged exposure to peak breeding levels; the other half of the males (C-males) received empty implants, thus maintaining normal levels of testosterone. Results demonstrated that experimentally enhanced testosterone increased male mating effort, as measured by song, courtship behaviour, home range size and success at obtaining extra-pair fertilizations (Ketterson *et al.* 1992; Chandler *et al.* 1994; Enstrom *et al.* 1997; Raouf *et al.* 1997; Reed *et al.* 2006), but that the increase came at a cost both to parental behaviour, as measured by nestling feeding rate and nest defence, and to self-maintenance, as measured by body mass, immune function and survival (Ketterson *et al.* 1991, 1992; Cawthorn *et al.* 1998; Schoech *et al.* 1998; Casto *et al.* 2001; Reed *et al.* 2006). An implication is that testosterone may mediate an adaptive trade-off between these functions. When fitness was modelled as relative potential for population growth, T-males had higher fitness than C-males, because the benefits of enhanced mating success outweighed the costs of reduced survival and parental care (Reed *et al.* 2006). The implication here is that seemingly fit possible phenotypes 'located' in the genome—those uncovered by experimental elevation of testosterone—are not normally expressed, and the ensuing question is why.

More recent research on juncos has addressed these implications. First, we asked whether natural variation in male testosterone does in fact underlie an adaptive resolution of the trade-off between mating effort and parental effort/survival at the level of individual variation. Second, we asked why natural counterparts to T-males are not more common in nature. Anticipating that selection on males might lead to correlated response in females, we asked whether male response to selection favouring expression of the full range of possible male phenotypes might be constrained by the detrimental impact that selection could have on females.

To address the first question, we assessed natural variation in male testosterone, focusing our attention on variation in undisturbed circulating levels and on male capacity to produce short-term testosterone increases. In many songbirds (including closely related sparrows), natural testosterone levels fluctuate rapidly in response to social stimuli such as competing males or potential mates (e.g. Wingfield 1985; Pinxten *et al.* 2003; Landys *et al.* 2007). Social modulation of circulating testosterone, which is the foundation of the 'challenge hypothesis', is thought to allow males to produce testosterone only when circumstances call for it, lessening the costs that would accompany constitutively high testosterone levels (Wingfield *et al.* 1987, 1990, 2001; Goymann *et al.* 2007). To assess variation among male juncos in their baseline levels of testosterone and their capacity to produce short-term testosterone increases, we used injections of gonadotropin-releasing hormone ('GnRH challenges') to stimulate the hypothalamic-pituitary-gonadal (HPG) axis to produce transient increases of plasma testosterone. Importantly, when males were challenged multiple times across the breeding season, the magnitude of the short-term testosterone increase (i.e. the difference between post- and pre-challenge levels) was found to be repeatable (Jawor *et al.* 2006). Although we have yet to measure heritability due to the difficulty of obtaining a large sample of relatives, individual consistency suggests the possibility of genetic variance underlying variation in short-term testosterone increases. GnRH challenge response is not only repeatable but also ecologically relevant, as the testosterone levels generated by GnRH challenges predicted levels produced in response to a male territorial intruder (McGlothlin *et al.* 2008).

To determine whether natural variation in testosterone is related to the trade-off between mating effort and parental effort, as predicted by the implant studies, we again used GnRH challenges and correlated the results with the results of standardized protocols to elicit mating-related and parental behaviour. As a measure of mating effort, we assessed territorial aggression in response to simulated territorial intrusions (Wingfield 1985). As a measure of parental effort, we assessed nestling feeding rate. With respect to aggression, we found that the peak testosterone levels produced in response to GnRH predicted a male's level of aggression toward the intruder, which indicates that males with more responsive HPG axes invest more effort in territorial defence (McGlothlin *et al.* 2007). With respect to parental behaviour, we found that males with larger changes in testosterone levels in response to GnRH made fewer trips to the nest during nestling feeding (McGlothlin *et al.* 2007). These correlations provide compelling evidence that natural

variation in testosterone production affects a fundamental life-history trade-off, thus confirming expectations generated by studies based on experimental testosterone elevation.

Critically, however, if we are to know whether the suite of traits mediated by testosterone is the adaptive outcome of correlational selection on the traits comprising the suite, we will need to relate response to GnRH to different components of fitness. This effort is currently underway. Future studies should also address two caveats. First, although the primary function of territorial defence is to maintain a breeding location, making it a reasonable proxy for mating effort, future studies should measure behaviours such as courtship to determine whether they too are related to testosterone in response to GnRH. Second, these studies correlating behaviour to response to GnRH were not conducted on the same individuals at the same time. Future studies should confirm that testosterone in response to GnRH covaries with territorial aggression and parental behaviour when all are measured in the same individuals.

The results also raise the important question of why individual males should vary in the resolution of mating effort/parental effort trade-off, and thus in testosterone production, at all. Another study suggests a possible reason why some males should be submissive and parental while others are aggressive and less parental. The magnitude of a male's testosterone increase in response to GnRH was found to be positively correlated with the size of the white plumage patch on its tail (McGlothlin *et al.* 2008). This patch, referred to as tail white, is displayed to females during courtship and other males during escalated aggressive encounters (Nolan *et al.* 2002). Females prefer males with larger white patches (Hill *et al.* 1999), and these males also tend to be socially dominant (Balph *et al.* 1979; Holberton *et al.* 1989). The correlation between tail white and testosterone, then, suggests that more attractive, dominant males tend to produce larger testosterone increases. Variation in the mating effort/parental effort trade-off may thus be linked to attractiveness.

Such an association could be maintained by correlational selection, which would arise if testosterone production and tail white interact in their effects on fitness. For example, it may be useless for a male to be attractive if he does not invest energy in the behaviours needed to obtain mates. Hence, mating behaviour and appearance are likely to interact in their effects on fitness, and attractive males that expend considerable effort may be highly successful at obtaining mates. As a correlate, however, unattractive males that spare investment in mating behaviour and focus on alternative routes to fitness may also benefit. Interactive fitness effects like this are likely to be common, and there may be many instances where correlational selection acts to associate mechanisms underlying trade-off resolution with attractive signals. Because there are several routes to obtaining fitness, this type of selection can also maintain variation in the resolution of trade-offs as well. As seen from this perspective, the suite of two behaviours and one plumage trait may act collectively as an adaptation allowing males to produce optimal levels of mating and parental effort depending on their attractiveness. If so, the absence of males similar to T-males may be explained by the frequency dependence of any advantage this phenotype might provide.

Up to this point, we have considered the evolution of testosterone-mediated suites from a solely male perspective. However, because males and females share much of their genome, male and female traits do not always follow completely independent evolutionary trajectories. Across species, mean testosterone levels of males and females tend to be correlated, suggesting that they may have coevolved (Wingfield 1994; Ketterson *et al.* 2005; Møller *et al.* 2005; Mank 2007). Within species, a genetic correlation across the sexes could act as a genetic constraint on the evolution of testosterone-mediated traits in males due to a correlated response to selection in females. To study this possibility, we examined the behavioural and fitness effects of experimentally elevated testosterone in females, and have begun preliminary studies of individual variation in females.

Studies of captive juncos have shown that females, like males, have decreased immune function when implanted with testosterone (Zysling *et al.* 2006). Whereas downregulation of the immune system by testosterone may be adaptive for males (because it allows them to divert energy to mate acquisition), it may represent a net cost for females (Zuk 1990). Also in captives, though not in free-living females, experimentally elevated testosterone inhibited brood patch formation (Clotfelter *et al.* 2004). In the wild, testosterone implantation seemed to interfere with nest initiation, as time to first egg was longer in T-females (Clotfelter *et al.* 2004). Incubation consistency and nest defence during the egg stage were unaffected (Clotfelter *et al.* 2004). Another captive study suggested that testosterone may interfere with mating decisions (McGlothlin *et al.* 2004).

It is not yet clear whether testosterone in females acts as an evolutionary constraint on the testosterone-mediated suite in males. The extent to which the sexes are genetically correlated in testosterone production is unknown. Our initial investigation of individual variation indicates that males and females may regulate testosterone in different ways. Whereas males respond to GnRH challenges throughout the breeding season, females seem to do so only when producing eggs (Jawor *et al.* 2007). This effect has interesting implications for the role of female testosterone in maternal effects, as the magnitude of this GnRH challenge response showed a strong correlation with testosterone deposited in the yolk, but it suggests that the evolution of the testosterone-mediated suite may be somewhat decoupled across sexes. Further work, especially applying the approaches described in §4 to both sexes, is necessary to determine the importance of cross-sexual interactions in the evolution of hormone-mediated suites.

6. CONCLUSION

Although there is a wealth of knowledge about variation in hormone profiles in natural populations and about the impact of experimentally altered hormones on suites of phenotypic characters, we are only just

beginning to dissect the mechanisms responsible for evolutionary change in hormonal pathways and hormone-mediated suites (Adkins-Regan 2005). Here, we have advocated integrating the methods and theory of quantitative genetics with traditional endocrinological approaches as a promising way to address this issue. When combined with other approaches, including molecular and developmental genetics, the synthesis appears likely to provide important insights as we strive to understand how the inside world of organisms becomes adapted to the outside world.

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