

Proximate mechanisms of behavioural inflexibility: implications for the evolution of personality traits

Renée A. Duckworth^{*,1} and Keith W. Sockman²

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA; and ²Department of Biology, Curriculum in Neurobiology, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA

Summary

1. Behaviour is often assumed to be the most flexible of traits, yet recent studies show a high repeatability of behaviour within individuals even across different functional contexts. Such consistent expression of behaviour may evolve either when selection favours its integration with less flexible components of the phenotype or when pleiotropic effects produce correlations between behaviours that have different optimal timing of expression. Examining the physiological mechanisms underlying correlated expression of behaviours provides powerful insight into the evolution of personalities by establishing the extent to which pleiotropic effects might limit the independent evolution of distinct behaviours.

2. Here, we investigated proximate mechanisms behind aggressive and non-aggressive personality types in western bluebirds, *Sialia mexicana*, to determine whether consistency in the expression of aggression is because of shared effects of plasma-circulating androgens on aggression and mating behaviour.

3. We found that androgen concentration was unrelated to variation in both intra- and interspecific aggression even though it was closely linked to variation in male mating behaviour. These results suggest that pleiotropic effects of circulating androgens are unlikely to cause consistent differences among individuals in aggression.

4. These findings suggest that decoupling of the activational effects of hormones on behaviour is an important step in the evolution of personality traits.

Key-words: aggression, evolutionary constraint, *Sialia*, testosterone

Introduction

Personality traits are ubiquitous among animals; however, their evolution remains poorly understood (Dall, Houston & McNamara 2004; Bell 2007; Bergmüller & Taborsky 2010). Particularly puzzling is the remarkable consistency in behaviour across contexts even when variable expression of behaviour between contexts confers higher fitness (Sih, Bell & Johnson 2004). Both adaptive and non-adaptive hypotheses that have been developed to explain such behavioural consistency are based on observations that personality traits are correlated in their expression with a suite of other behavioural and life-history components of the phenotype. Adaptive consistency in the expression of behaviour might then evolve when selection favours its integration with other, less flexible traits or strategies (Dall, Houston & McNamara 2004; Carere & Eens 2005; Wolf *et al.* 2007).

Under this scenario, consistency in behaviour is adaptive when considering an individual's overall life-history strategy even though it may lead to a fitness loss in the short term or in a limited set of contexts. Alternatively, consistency in behaviour may instead reflect an evolutionary constraint if behaviours that have distinct optimal timing of expression are unable to vary independently because of pleiotropic gene effects (Ketterson & Nolan 1999; Adkins-Regan 2008; McGlothlin & Ketterson 2008; Duckworth 2010). Investigating the proximate mechanisms behind personality variation can provide insight into their evolution because it allows us to establish whether correlated expression of different behaviours is regulated by the same physiological mechanisms, and thus the extent to which pleiotropic effects might influence their expression and limit their independent evolution (Sih, Bell & Johnson 2004; Careau *et al.* 2008).

Hormones commonly integrate distinct behaviours either through organizational effects, when they act during early

*Correspondence author. E-mail: rad3@email.arizona.edu

ontogeny and influence brain anatomy and neurochemistry and determine the distribution of hormone receptors or through activational effects during the adult stage, such as when a surge of hormone elicits a specific behavioural response in a particular context (Moore 1991; Rhen & Crews 2002). In general, activational effects of hormones are expected to be prevalent in regulation of the expression of flexible phenotypes, whereas organizational effects set the stage for permanent behavioural differences among individuals (Ball & Balthazart 2008; Duckworth 2010). Thus, adaptive and non-adaptive hypotheses for the evolution of personality make distinct predictions about the importance of activation vs. organizational effects of hormones as determinants of personalities. If personality variation is adaptive and selection has favoured highly consistent expression of behaviour, then activational effects of hormones should be decoupled from variation in the expression of behaviour (Hau 2007; Duckworth 2010). However, under the non-adaptive scenario, behavioural consistency reflects the difficulty (over an evolutionary time-scale) of severing the link between hormone signalling and an integrated suite of behavioural responses. Under this scenario, activational effects of hormones persist even though the observed behavioural phenotype shows very low levels of flexibility.

In this study, we test these alternative hypotheses by investigating whether variation in androgen expression during adulthood (activational effects) underlies variation in aggressive behaviour of male western bluebirds (Fig. 1; *Sialia mexicana*) and integrates aggression with male mating behaviour. In this species, aggressive males attain preferred territories (Duckworth 2006a), invest less in parental care (Duckworth 2006b) and have a higher propensity to disperse compared with non-aggressive males (Duckworth & Badyaev 2007). Moreover, aggression is highly consistent across breeding stages (e.g. egg laying vs. incubation), distinct behavioural contexts (e.g. intra- vs. interspecific territory defence; Duckworth 2006b) and years of life (Duckworth & Badyaev 2007).



Fig. 1. Male (left) and female (right) western bluebirds (*Sialia mexicana*). Photo by Alex Badyaev.

The link between aggression and dispersal is adaptive in this species because aggressive individuals have the highest fitness when dispersing and colonizing new habitat patches where interspecific competition is high, whereas non-aggressive males have the highest fitness when remaining in their natal population where they obtain territories adjacent to their relatives (Duckworth 2008). However, within population comparisons show that aggressive males have lower fitness than non-aggressive males because they invest less in parental care (Duckworth 2006b); this fitness cost is most evident in older habitat patches where breeding density is highest (Duckworth 2008). Given the cost of aggression, individuals that disperse should remain aggressive while competing for a new territory, but decrease their aggression and focus on parental investment once they have acquired a territory. Thus, even though the link between aggression and dispersal is favoured in some contexts, it is unclear whether observed consistency in the expression of aggression across all contexts throughout an individual's life is adaptive. On the one hand, it may be adaptive if it is necessary to enable integration of aggression and dispersal. On the other hand, consistency in the expression of aggression may not be related to its integration with dispersal and may instead be due to pleiotropic effects of androgens that influence the expression of aggression and a suite of other behavioural traits.

Links between aggression, mating behaviour and parental care are common in birds because androgens, which stimulate spermatogenesis, frequently mediate variation in aggression and are antagonistic to the expression of parental behaviour (Wingfield *et al.* 1990; Ketterson & Nolan 1994). Thus, aggressive personalities of western bluebirds may be due to the pleiotropic effects of androgens that regulate distinct mating tactics of males. Under this scenario, consistently high androgen concentrations are necessary for males to pursue extra-pair copulations, and, as a side effect, cause consistently high aggression across contexts. This hypothesis predicts that more aggressive males should pursue extrapair mating opportunities more often and should have consistently higher androgen levels compared with non-aggressive males. Alternatively, if selection has favoured the context-independent expression of aggression (e.g. to enable its integration with dispersal behaviour), then aggressive personality variation should be unrelated to androgen concentration in adulthood. We tested these hypotheses by determining whether variation in baseline circulating androgen concentration of male western bluebirds is associated with aggressive personality type and mating behaviour.

Materials and methods

STUDY SYSTEM AND GENERAL METHODS

Western bluebirds are obligate secondary cavity nesters, and nest cavities are limited in the environment leading to intense intra- and interspecific aggressive interactions over nest sites (Guinan, Gowaty & Eltzroth 2000). Although western bluebirds are socially monogamous, they have significant levels of extrapair mating behaviour – in

the study population, approximately 40% of nests have extrapair offspring and 12% of all offspring are sired by extrapair fathers (Duckworth 2006b).

For this study, we collected data in 2004–2005 at a nest-box population of banded western bluebirds in western Montana (see Duckworth 2006a for a detailed description of the study site). Nest boxes were visited weekly from April through July to monitor the progress of nests, collect blood samples from nestlings and to determine pairing and nesting affiliations of breeding adults. Resident birds were captured using traps baited with mealworms to collect a blood sample for hormone and paternity analysis and to mark them with a unique colour band combination and take standard morphological measurements. Males were categorized as either second year (i.e. in their first year of breeding) or after second year based on banding records and moult status of their greater secondary wing coverts (Shizuka & Dickinson 2005).

HORMONE SAMPLING AND MEASUREMENT

We obtained samples for hormone analysis from 55 breeding males (39 and 16 during the territory acquisition and parental care stages, respectively) by collecting 50–150 μL of blood by brachial venipuncture within 10 min of capture. Blood was stored on ice in the field until it could be spun in a microhematocrit centrifuge to separate the plasma and red blood cells, which were then stored at -80°C . Plasma androgen levels were determined in three assays, which were run in either duplicate or triplicate using a testosterone Enzyme immunoassay (EIA) assay from Cayman Chemical (<http://www.caymanchem.com>). Samples were randomized across the plates with respect to male aggression, capture date and measures of paternity. This assay uses a 96-well plate coated with testosterone-specific rabbit antiserum binding sites and has specificity to testosterone of 100%, 5α -dihydro testosterone of 27.4%, 5β -dihydro testosterone of 18.9% and $< 5\%$ for androstenedione, 11-keto testosterone and 5-androstenediol. Serial dilutions of pooled plasma from breeding male western bluebirds demonstrated strong binding to the antiserum coated plates – the slope of the curve for western bluebird plasma ($F = 217.48$, $P < 0.01$, $b_{\text{ST}} = -0.98$) was very similar to the slope of the standard used in the kit ($F = 78.24$, $P < 0.01$, $b_{\text{ST}} = -0.96$). Moreover, to test for accuracy, an aliquot of the pooled plasma was stripped of endogenous steroid using dextran-coated charcoal and then was spiked with 500 pg mL^{-1} of testosterone standard. The recovery for this sample was 84%, which was slightly higher than our average extraction efficiency, indicating that this kit could be used to assess testosterone concentrations in western bluebirds accurately. Samples were purified with ether extraction and were run in three assays according to the kit protocol. Extraction efficiency was determined by including a plasma sample that was first stripped of hormone using a charcoal solution and spiked with a known amount of the testosterone standard. This sample was used in all assays to determine the interassay variation. Mean intrassay variation was 11.9%, interassay variation was 16.8%, average extraction efficiency was 74.8% and assay detection limit was 6 pg mL^{-1} .

MICROSATELLITE GENOTYPING AND PATERNITY ANALYSIS

Nestlings and adults were genotyped at four polymorphic microsatellite loci $\text{Cu}\mu$ 02, $\text{Cu}\mu$ 04, Ssi 8–19 and Ssi 9–32 (Duckworth 2006b). Parentage was assessed for each nest by comparing genotypes of offspring and the attending adults. All females matched their offspring's

genotype, and the attending male was excluded as the father if one or more loci did not match. Extrapair offspring were assigned to a sire if their genotypes matched completely, and there was no other male in the population with a matching genotype. In three cases where there were two males that matched the genotype of an extrapair nestling, we assigned paternity to the male whose breeding territory was closest because previous work has shown that neighbours are the most common sires of extrapair offspring (R. A. Duckworth, unpublished data). Out of 304 offspring, 11.8% were extrapair and over 98% of these extrapair offspring were assigned to a sire.

MEASUREMENT OF INTRA- AND INTERSPECIFIC AGGRESSION

Following a previously established protocol (Duckworth 2006b), interspecific aggression of all males was measured in response to a simulated territorial intrusion by a tree swallow (*Tachycineta bicolor*). For a subset of these males in 2004, intraspecific aggression was also measured in response to a simulated territorial intrusion of a conspecific male. Aggression towards a conspecific was only measured for a subset of individuals because intra- and interspecific aggression positively correlated and because simulating a territorial intrusion by a conspecific male was disruptive to breeding (it led to three cases of infanticide by the focal male). Moreover, bluebirds' response to a tree swallow is biologically meaningful in a territorial context because it is closely linked to territory acquisition (with more aggressive males gaining larger- and higher-quality territories) and bluebirds are significantly more aggressive towards tree swallows compared with resident non-cavity nesting species (Duckworth 2006b; Duckworth & Badyaev 2007). Birds used as intruders were captured at sites 30–50 km away to avoid any prior interactions influencing the focal individual's behaviour and different intruder individuals were used in each trial. For the subset of males measured for intraspecific aggression, the order of the intruder presentations were randomized such that seven males were presented with a bluebird first and five were presented with a tree swallow first. These trials were conducted on consecutive days whenever possible but no more than one day separated each trial. Briefly, to simulate territorial intrusions, pairs were presented with either a live swallow or male western bluebird in a wire cage placed on the nest box. We recorded the number of times the focal individual attacked, flew by or hovered near the intruder during a two-minute period starting from the focal bird's approach. Behaviours were summarized into an aggression score that varied from 1 to 6 with 1 indicating the least aggressive response and 6 the most aggressive response. Scores were assigned according to the following scale: 1: no aggressive behaviours, 2: hovering or flying by 1–5 times and 0 attacks, 3: hovering or flying by more than 5 times and 0 attacks, 4: 1–5 attacks, 5: 6–9 attacks, and 6: 10 or more attacks. Both types of aggression were measured within a week of nest initiation and for individuals that bred at the study site in both years, interspecific aggression was measured across years as well. All procedures were conducted according to protocol A090-04-03 approved by the Duke University Institutional Animal Care and Use Committee (IACUC).

MEASURING EXTRAPAIR MATING EFFORT

Although aggression is not associated with acquisition of extrapair paternity in western bluebirds (Duckworth 2006b), it is still possible that aggressive males pursue extrapair mating opportunities more often than non-aggressive males, but are simply not successful. Thus,

we used three kinds of data as a proxy for male pursuit of extrapair matings: (i) the per cent of time a male spent off his territory during incubation, (ii) whether a male sired extrapair offspring, and (iii) whether a male was cuckolded. The first measure was calculated for a subset of nests ($N = 13$) in which we directly observed territories during incubation using a blind. Western bluebirds are highly territorial and rarely venture openly onto neighbouring territories (e.g. to forage) because this results in aggressive disputes with territory owners. Moreover, territories are open and are small enough (< 150 m wide) that it is easy to locate the birds on their territories. Based on these observations, we assumed that males that were not observed on their territories during focal observations were absent because they were pursuing extra-pair mating opportunities as has been shown in other passerine species (e.g. Stutchbury *et al.* 2005; Evans, Stutchbury & Woolfenden 2008). Nesting is asynchronous in this species, and there were always at least one or more fertile females on nearby territories during these incubation watches. For the second measure of extrapair mating activity, males that successfully sired extrapair offspring were assumed to have pursued extrapair mating opportunities more often than males that did not sire extrapair offspring. This measure may not be concordant with the other two measures depending on how often males that pursue extrapair matings are successful. Finally, other studies have shown that there is a trade-off between seeking extra-pair copulations and mate guarding (MacDougall-Shackleton, Robertson & Boag 1996; Weatherhead & Yezerinac 1998; Saino *et al.* 1999; van de Crommenacker *et al.* 2004); therefore, for the third measure, we assumed that if a male was cuckolded, he was away from his territory pursuing extrapair mating opportunities more often than a male that was not.

STATISTICAL ANALYSES

To investigate changes in androgen levels across breeding stages, we classified males into the 'territory acquisition' stage if they were sampled before incubation onset and into the 'parental' stage if they were sampled during incubation or nestling stage.

Because the hypothesis we were testing explicitly predicts that males with aggressive personalities should have consistently higher androgen levels than non-aggressive males throughout the breeding period and because excluding males captured during incubation and nestling stages did not influence the results, we included all males in analyses, regardless of the stage at which they were captured. Log-transformed androgen concentration was correlated with capture date ($F_{(1, 54)} = 27.85$, $b = -0.59$, $P < 0.01$); therefore, to control for the potentially confounding effect of seasonal androgen changes (Sockman, Schwabl & Sharp 2004), we used the residuals of a regression of log-transformed androgen levels and capture date in analyses investigating the influence of androgens on behavioural traits. Because many studies have found that testosterone is related to aggression only during periods of social instability (e.g. Wingfield *et al.* 1990; Goymann, Landys & Wingfield 2007), we also analysed the relationship between androgen concentration and intra- and interspecific aggression for the subset of individuals for which hormone samples were collected early in the season during the period when territorial disputes were common (from mid-March to early May). We used 5th of May as the cut-off point for this analysis because $< 3\%$ of territorial disputes were observed after this date (R.A. Duckworth, unpublished data).

Eleven males were sampled in both 2004 and 2005 enabling us to calculate repeatability of androgen concentration and aggressive behaviour across years using ANOVA with individual identity as the

independent variable (Lessells & Boag 1987). Because of these repeated measures across years, we used mixed models with individual identity and year fitted as a random effect to analyse the relationship between androgen concentration and interspecific aggression. We report the parameter estimate (b) and standard error from this model. We used a generalized linear model with a binomial error structure and logit-link function to determine whether androgen concentration differed among males that were categorized by whether or not they were cuckolded or had acquired extrapair paternity. We also included male age, year and their interaction to control for these potentially important effects and deleted terms that were not significant from all final models. We ran these analyses first with the entire data set and then by randomly deleting one repeated measure for males that were sampled in both years. Randomly deleting repeated measures of males did not affect the results and therefore, we report results using the entire data set here. To assess the size of the effect of androgen concentration on male-mating behaviour, we calculated Cohen's D using the pooled standard deviation. Sample sizes for analyses involving measures of extrapair mating activity and aggression vary depending on individual nest success as we were unable to acquire DNA samples from a few offspring because of nest predation. We could not analyse the relationship between intraspecific aggression and the amount of time a male spent off his territory because we completed incubation watches for very few males measured for this trait.

Results

ANDROGEN CONCENTRATION AND MATING BEHAVIOUR

Androgen concentration differed across breeding stages – males that were sampled prior to incubation onset had higher androgen levels (mean \pm SE = 2711.18 ± 407.11 pg mL⁻¹) than males sampled during the parental care stages (mean \pm SE = 533.39 ± 137.51 pg mL⁻¹; $F_{(1, 54)} = 10.45$, $P < 0.01$; Fig. 2a), but did not differ across years ($t = -1.60$, $P = 0.12$, $N = 55$). Androgen concentration was not repeatable across years ($F_{(10, 21)} = 0.44$, $R < 0.01$, $P = 0.90$). Males with higher androgen levels engaged in extrapair mating behaviour more frequently – they were more likely to gain extrapair fertilizations ($\chi^2 = 7.63$, $P < 0.01$, $d = 0.86$, $N = 53$; Fig. 2b), were cuckolded more often ($\chi^2 = 6.10$, $P = 0.01$, $d = 0.85$, $N = 46$; Fig. 2c), and they spent more time off their territories during incubation ($t = 3.42$, $P < 0.01$, $N = 13$; Fig. 3a) compared to males with lower androgen levels. These results were robust even after taking into account the potentially confounding factors of age-related ($\chi^2 = 6.94$, $P < 0.01$) and annual ($\chi^2 = 4.91$, $P = 0.03$) variation in the propensity of males to gain extrapair paternity. Older males were more likely to gain extrapair paternity than younger males (47.6% of after second year males vs. 9.4% of second year males gained extrapair paternity) and also had higher androgen levels ($t = 1.98$, $P = 0.05$); however, *post-hoc* analysis showed that the correlation between male age and androgen variation was not significant when extrapair mating behaviour was taken into account (Mixed model, $t = 0.80$, $P = 0.45$), suggesting that androgen variation is only indirectly related to age. Age did

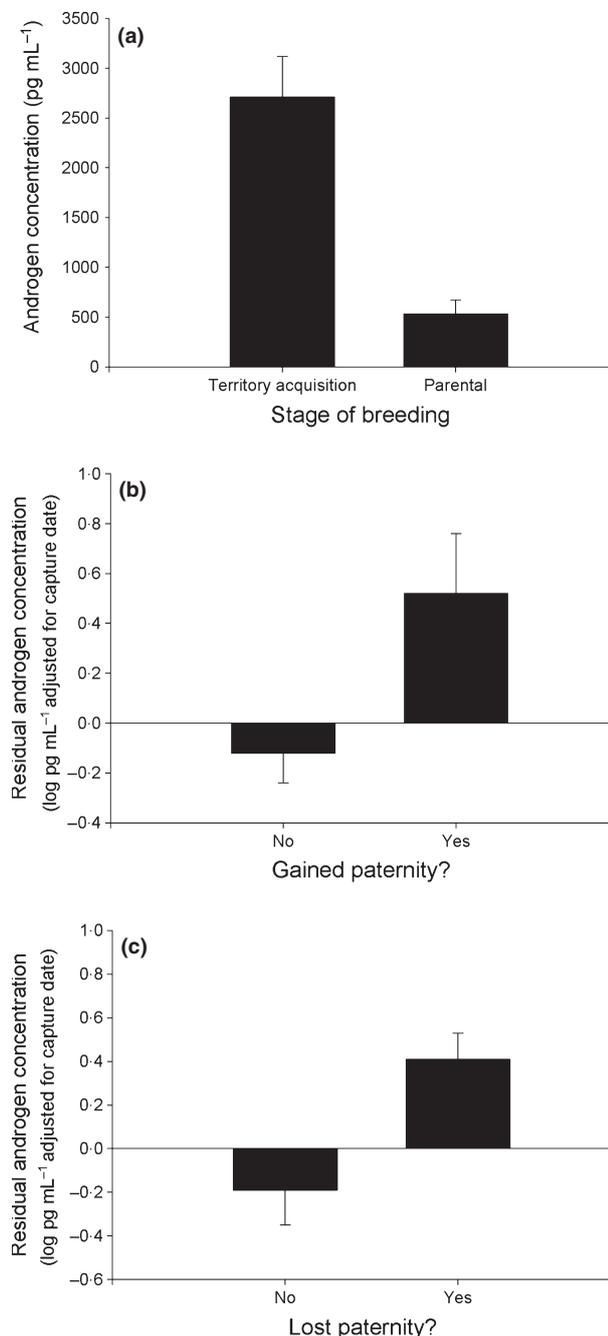


Fig. 2. Androgen concentration in relation to breeding stage and extrapair paternity. (a) Males sampled during the territory acquisition stage (before incubation onset) had higher androgen levels than males sampled during the parental care stages (incubation and nestling stages). (b) Males that gained extrapair paternity had higher residual androgen levels (measured as the residuals of a regression of androgen elevation and capture date) compared with males that did not. (c) Males that lost paternity in their own nests had higher residual androgen levels compared with males that did not.

not influence whether a male lost paternity in his own nest ($\chi^2 = 1.24$, $P = 0.27$) or the amount of time a male spent off his territory ($t = 1.08$, $P = 0.31$). There was no association between paternity lost and paternity gained ($\chi^2 = 0.28$, $P = 0.60$, $N = 46$).

ANDROGEN CONCENTRATION AND VARIATION IN AGGRESSION

Males sampled across years showed high repeatability of aggression ($F_{(10, 21)} = 2.94$, $R = 0.96$, $P = 0.04$). Androgen concentration was not related to variation in either intra- or interspecific aggressive behaviour (Intraspecific: $F = 0.22$, $b = 0.15$, $P = 0.65$, $N = 11$; Interspecific: $F = 0.05$, $b = -0.03 \pm 0.16$, $P = 0.83$, $N = 54$; Fig. 3b,c). Confining the analysis to the period of social instability did not alter this result (Intraspecific: $F = 0.19$, $b = 0.17$, $P = 0.67$, $N = 9$; Interspecific: $F = 0.05$, $b = 0.05 \pm 0.02$, $P = 0.86$, $N = 31$). In some species, androgen concentration spikes and can remain elevated after individuals experience a territorial intrusion (Wingfield *et al.* 1990); however, we found no evidence that simulated territorial intrusions biased our analyses. The few males that were sampled within a week following simulated territorial intrusions ($N = 7$) did not have higher androgen levels compared with males sampled before simulated territorial intrusions ($N = 33$; T -test: $t = -0.28$, $P = 0.78$). Moreover, limiting the analysis of aggression and androgen concentration to males that were sampled before simulated territorial intrusions did not affect the results ($t = -0.26$, $P = 0.81$, $N = 33$).

AGGRESSION AND EXTRAPAIR MATING BEHAVIOUR

Aggression was not related to extrapair mating effort; aggressive males did not spend more time off their territories than non-aggressive males (Interspecific: $t = -0.65$, $P = 0.53$, $N = 13$), were not cuckolded more often than non-aggressive males (Intraspecific: $t = 0.38$, $P = 0.72$, $N = 10$; Interspecific: $t = 0.02$, $P = 0.98$, $N = 52$) and did not gain extrapair fertilizations more often than non-aggressive males (Intraspecific: $t = 0.37$, $P = 0.73$, $N = 10$; Interspecific: $t = 0.99$, $P = 0.35$, $N = 45$).

Discussion

Consistent differences among individuals in behaviour could reflect adaptive specialization to a particular life-history strategy or could be caused by an underlying hormonal constraint. If consistency in the expression of behaviour is adaptive, then we expect activational effects of hormones, which commonly regulate flexible phenotypic responses, to be decoupled from variation in behaviour (Moore 1991; Ball & Balthazart 2008; Duckworth 2010). However, hormone-behaviour links may persist if there hasn't been enough time for selection to break up co-evolving complexes of traits (Ketterson & Nolan 1999; Hau 2007; McGlothlin & Ketterson 2008). Thus, understanding the proximate mechanisms underlying variation in personality traits can provide insight into potential constraints that shape variation in behaviour.

Our study of the hormonal basis of aggressive behaviour in western bluebirds produced three main results. First, we found that natural variation in circulating androgen levels was not associated with variation in either intra- or interspecific

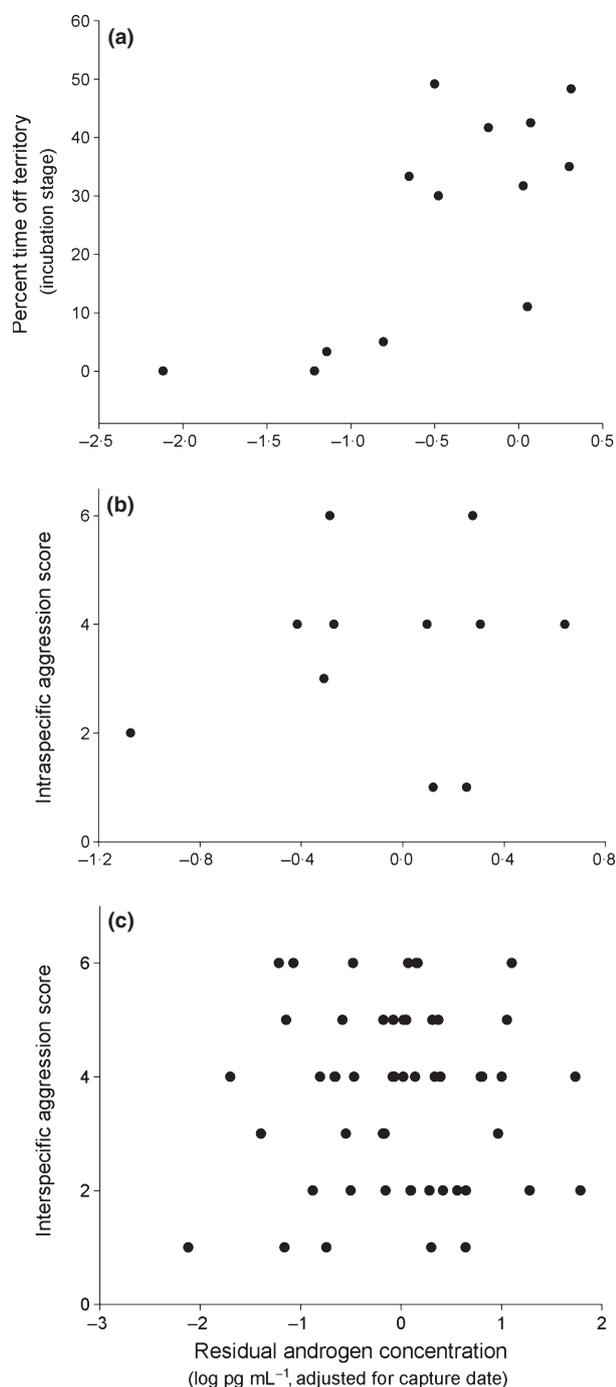


Figure 3. (a) Androgen concentration was positively related to the amount of time males spent off their territory during the incubation stage. Residual androgen concentration was not related to either (b) intraspecific or (c) interspecific male aggression score.

aggressive behaviour. Second, we found that androgen levels were closely associated with male extrapair mating behaviour. Using three different measures, we showed that males that pursued extrapair mating opportunities were more likely to have higher androgen levels compared with males that did not. Third, we found that variation in male aggression was not related to male-mating behaviour. Taken together, these findings reject the hypothesis that variation in aggressive

behaviour is due to an androgen-based hormonal constraint in western bluebirds. The hormonal constraint hypothesis specifically predicts that aggressive males will have consistently higher androgen concentrations compared with non-aggressive males. Not only was there no association between natural variation in androgen concentration and either intra- or interspecific aggressive behaviour, but in males that were sampled across years, androgen concentration was not significantly repeatable even though aggression was highly repeatable across years. These findings raise several questions. How is variation in aggressive behaviour determined in this species? Why was androgen level closely associated with extrapair mating behaviour, but not with aggression? And why do individuals display consistent differences in aggressive behaviour?

Although we found no link between circulating androgens (activational effects) and variation in either intra- or interspecific aggressive behaviour, androgens may still be important in determining male aggressive personality through their organizational effects (Caro & Bateson 1986; Moore 1991; Crews 1998). Indeed, variation in aggression among individuals is partly due to maternal effect; egg-laying order is linked to offspring aggression such that, within families, earlier produced males are more aggressive in adulthood compared with later produced males (Duckworth 2009). While the mechanisms underlying this maternal effect are not known, a hormonal gradient across the egg-laying order may induce differences in male aggressive phenotype. In birds, early developmental effect of hormones on morphology and behaviour (Schwabl 1996; Forstmeier, Coltman & Birkhead 2004; Groothuis *et al.* 2004; Strasser & Schwabl 2004; Carere & Balthazart 2007; Sockman *et al.* 2008) and hormonal gradients across the egg-laying order (Williams *et al.* 2005; Badyaev *et al.* 2008) are common. These observations, in combination with studies in humans and lab animals that demonstrate a link between personality variation and hormone exposure early in ontogeny (Carere & Balthazart 2007; Hines 2008), suggest that organizational effects of hormones may be more important than their activational effects as determinants of personality variation.

The results of this study corroborate recent findings that the regulation of aggression by androgens is evolutionarily labile (reviewed in Hau, Gill & Goymann 2008). This raises the question of whether such evolutionary lability is common for androgen-dependent traits in general. In birds, secondary sexual traits and behaviours such as aggression, plumage colour and song rate are dependent on androgens in some species, but not in others (e.g. Eens *et al.* 2000; Peters *et al.* 2000; Badyaev & Hill 2003; Duckworth, Mendonça & Hill 2004; Day, McBroom & Schlinger 2006; Lynn & Wingfield 2008). However, primary sexual traits, such as spermatogenesis, appear to be universally androgen dependent in vertebrates (Miura *et al.* 1991; Holdcraft & Braun 2004). Thus, one possible explanation for differences in androgen dependence in behaviour across species is that traits are androgen dependent when their expression overlaps temporally with gonadal development and spermatogenesis. A particularly good

illustration of this idea is mating behaviour, which, in most species, closely overlaps with spermatogenesis and is androgen dependent; however, exceptions to this rule are found in species that mate upon arousal from torpor such as the big brown bat (*Eptesicus fuscus*) and the red-sided garter snake (*Thamnophis sirtalis*). In these species, spermatogenesis is temporally dissociated from mating behaviour; mating occurs when the gonads are regressed and is stimulated by the perception of temperature changes rather than sex steroids (Crews 1984; Mendonça *et al.* 1996). In another example, birds which are territorial in the non-breeding season when gonads are regressed also show dissociation between androgens and aggression (e.g. Schwabl & Kriner 1991; Soma, Tramontin & Wingfield 2000). Finally, in tropical species of birds which display year-round territoriality, testosterone levels are generally low throughout the year even though territoriality is high. Instead, peaks in testosterone levels in these species are more closely aligned to periods of peak mating (Hau, Gill & Goymann 2008). These studies suggest that androgen dependence of secondary sexual traits (such as mating behaviour and aggression) is evolutionarily labile and may depend on the temporal overlap between expression of specific behaviours and spermatogenesis. This hypothesis may explain why there was a link between androgen concentration and extrapair mating behaviour, but not aggression in western bluebirds.

While this study has ruled out hormonal constraint as a primary cause of variation in aggressive personalities, we are still left with the question of why individuals display consistent differences in aggressive behaviour. One possibility is that selection for functional integration of aggression and dispersal (Duckworth 2008) has limited flexibility of aggression to ensure close coordination between these behaviours. In western bluebirds, the decision to disperse occurs early in an individual's life, possibly just weeks after fledging (Guinan, Gowaty & Eltzroth 2000). This early expression of dispersal behaviour, in combination with strong selection for coexpression of aggression and dispersal (Duckworth 2008), may favour early determination of aggression as well which may then limit flexibility in its expression later in life. Uncovering the developmental mechanisms underlying individual variation in both aggression and dispersal will be necessary to test this idea.

This study adds to a growing body of evidence that the link between androgens and aggressive behaviour is not straightforward. In a recent review, Ball & Balthazart (2008) note that previous attempts to correlate individual variation in aggressive behaviour to androgen levels have largely failed except during periods of fast change in behaviour, such as during periods of social instability. One potential explanation is that androgens may be more important in regulating the initial responsiveness of an individual to an intruder than the level of aggression displayed once they respond. We focused on the level of aggressive response in this study, leaving open the possibility that, in western bluebirds, other measures of aggression such as individual responsiveness to intruders are androgen dependent.

Studies of the proximate mechanisms underlying personality variation are a first step in understanding the relative importance of constraints and selection in shaping behavioural variation. Our study of the hormonal basis of variation in aggressive personality of western bluebirds shows that activational effects do not impose constraints on the evolution of behavioural flexibility; instead, personality types in this species may have evolved in response to selection for reliable coupling of aggression and dispersal. Future studies of the possible role of organizational effects of hormones on behaviours are likely to shed new light on the proximate mechanisms of personality variation.

Acknowledgements

We thank three anonymous reviewers Jennifer Grindstaff, Wolfgang Forstmeier, Alex Badyaev, Anne Storey, Virginia Belloni, Erin Morrison and Jordan Veal for their comments on earlier versions of this manuscript. We are grateful to Creagh Breuner and Judith Easterbrook for advice on using the EIA kits and Sabrina Burmeister and Charles Mitchell for use of lab equipment. We also thank Mukta Chakraborty for help with running the EIAs and members of Mountain Bluebird Trails and residents of the Hayes Creek neighbourhood for kindly allowing us to monitor nests on their properties. This work was supported by National Science Foundation grants (IRFP – 0601751 and DEB 918095 to RAD).

References

- Adkins-Regan, E. (2008) Do hormonal control systems produce evolutionary inertia? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **363**, 1599–1609.
- Badyaev, A.V. & Hill, G.E. (2003) Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 27–49.
- Badyaev, A.V., Young, R.L., Hill, G.E. & Duckworth, R.A. (2008) Evolution of sex-biased maternal effect in birds: IV. Intra-ovarian growth dynamics can link sex-determination and sex-specific acquisition of resources. *Journal of Evolutionary Biology*, **21**, 449–460.
- Ball, G.F. & Balthazart, J. (2008) Individual variation and the endocrine regulation of behaviour and physiology in birds: a cellular/molecular perspective. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **363**, 1699–1710.
- Bell, A.M. (2007) Future directions in behavioural syndromes research. *Proceedings of the Royal Society B*, **274**, 755–761.
- Bergmüller, R. & Taborsky, M. (2010) Animal personality due to social niche specialisation. *Trends in Ecology & Evolution*, **25**, 504–511.
- Careau, V., Thomas, D., Humphries, M. & Réale, D. (2008) Energy metabolism and animal personality. *Oikos*, **117**, 641–653.
- Carere, C. & Balthazart, J. (2007) Sexual versus individual differentiation: the controversial role of avian maternal hormones. *Trends in Endocrinology and Metabolism*, **18**, 73–80.
- Carere, C. & Eens, M. (2005) Unravelling animal personalities: how and why individuals consistently differ. *Behaviour*, **142**, 1149–1157.
- Caro, T.M. & Bateson, P. (1986) Organization and ontogeny of alternative tactics. *Animal Behaviour*, **34**, 1483–1499.
- Crews, D. (1984) Gamete production, sex steroid secretion and mating behavior uncoupled. *Hormones and Behavior*, **18**, 29–41.
- Crews, D. (1998) On the organization of individual differences in sexual behavior. *American Zoologist*, **38**, 118–132.
- van de Crommenacker, J., Richardson, D.S., Groothuis, T.G.G., Eising, C.M., Dekker, A.L. & Kondeur, J. (2004) Testosterone, cuckoldry risk and extrapair opportunities in the Seychelles warbler. *Proceedings of the Royal Society of London. Series B*, **271**, 1023–1031.
- Dall, S.R.X., Houston, A.I. & McNamara, J.M. (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters*, **7**, 734–739.
- Day, L.B., McBroom, J.T. & Schlinger, B.A. (2006) Testosterone increases display behaviors but does not stimulate growth of adult plumage in male golden-collared manakins (*Manacus vitellinus*). *Hormones and Behavior*, **49**, 223–232.

- Duckworth, R.A. (2006a) Aggressive behavior affects selection on morphology by determining the environment of breeding in a passerine bird. *Proceedings of the Royal Society of London. Series B*, **273**, 1789–1795.
- Duckworth, R.A. (2006b) Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behavioral Ecology*, **17**, 1011–1019.
- Duckworth, R.A. (2008) Adaptive dispersal strategies and the dynamics of a range expansion. *American Naturalist*, **172**, S4–S17.
- Duckworth, R.A. (2009) Maternal effects and range expansion: a key factor in a dynamic process? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **364**, 1075–1086.
- Duckworth, R.A. (2010) Evolution of personality: developmental constraints on behavioral flexibility. *The Auk*, **127**, 752–758.
- Duckworth, R.A. & Badyaev, A.V. (2007) Coupling of aggression and dispersal facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences USA*, **104**, 15017–15022.
- Duckworth, R.A., Mendonça, M.T. & Hill, G.E. (2004) Condition dependent sexual traits and social dominance in the house finch. *Behavioral Ecology*, **15**, 779–784.
- Eens, M., Duyse, E.V., Berghman, L. & Pinxten, R. (2000) Shield characteristics are testosterone-dependent in both male and female moorhens. *Hormones and Behavior*, **37**, 126–134.
- Evans, M.L., Stutchbury, B.J.M. & Woolfenden, B.E. (2008) Off-territory forays and genetic mating system of the wood thrush (*Hylocichla mustelina*). *The Auk*, **125**, 67–75.
- Forstmeier, W., Coltman, D.W. & Birkhead, T.R. (2004) Maternal effects influence the sexual behavior of sons and daughters in the zebra finch. *Evolution*, **58**, 2574–2583.
- Goymann, W., Landys, M.M. & Wingfield, J.C. (2007) Distinguishing seasonal androgen responses from male-male androgen responsiveness – Revisiting the Challenge Hypothesis. *Hormones and Behavior*, **51**, 463–476.
- Groothuis, T.G.G., Müller, W., von Engelhardt, N., Carere, C. & Eising, C. (2004) Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioural Reviews*, **29**, 329–352.
- Guinan, J.A., Gowaty, P.A. & Eltzroth, E.K. (2000) Western Bluebird (*Sialia mexicana*). *The Birds of North America, No. 510* (eds A. Poole & F. Gill), pp. 31. Birds of North America Inc., Philadelphia, PA.
- Hau, M. (2007) Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *Bioessays*, **29**, 133–144.
- Hau, M., Gill, S.A. & Goymann, W. (2008) Tropical field endocrinology: ecology and evolution of testosterone concentrations in male birds. *General and Comparative Endocrinology*, **157**, 241–248.
- Hines, M. (2008) Early androgen influences on human neural and behavioural development. *Early Human Development*, **84**, 805–807.
- Holdcraft, R. & Braun, R. (2004) Hormonal regulation of spermatogenesis. *International Journal of Andrology*, **27**, 335–342.
- Ketterson, E.D. & Nolan, V. (1994) Male parental behavior in birds. *Annual Review of Ecology and Systematics*, **25**, 601–628.
- Ketterson, E.D. & Nolan, V. (1999) Adaptation, exaptation, and constraint: a hormonal perspective. *The American Naturalist*, **154**, S4–S24.
- Lessells, C.M. & Boag, P.T. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk*, **104**, 116–121.
- Lynn, S.E. & Wingfield, J.C. (2008) Dissociation of testosterone and aggressive behavior during the breeding season in male chestnut-collared longspurs, *Calcarius ornatus*. *General and Comparative Endocrinology*, **456**, 181–189.
- MacDougall-Shackleton, E.A., Robertson, R.J. & Boag, P.T. (1996) Temporary male removal increases extra-pair paternity in eastern bluebirds. *Animal Behaviour*, **52**, 1177–1183.
- McGlothlin, J.W. & Ketterson, E.D. (2008) Hormone-mediated suites as adaptations and evolutionary constraints. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **363**, 1611–1620.
- Mendonça, M.T., Chernetsky, S.D., Nester, K.E. & Gardner, G.L. (1996) Effects of gonadal sex steroids on sexual behavior in the big brown bat, *Epptesicus fuscus*, upon arousal from hibernation. *Hormones and Behavior*, **30**, 153–161.
- Miura, T., Yamauchi, K., Takahashi, H. & Nagahama, Y. (1991) Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel (*Anguilla japonica*). *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 5774–5778.
- Moore, M.C. (1991) Application of organization-activation theory to alternative male reproductive strategies: a review. *Hormones and Behavior*, **25**, 154–179.
- Peters, A., Astheimer, L.B., Boland, C.R.J. & Cockburn, A. (2000) Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, *Malurus cyaneus*. *Behavioral Ecology and Sociobiology*, **47**, 438–445.
- Rhen, T. & Crews, D. (2002) Variation in reproductive behaviour within a sex: neural systems and endocrine activation. *Journal of Neuroendocrinology*, **14**, 517–531.
- Saino, N., Primmer, C.R., Ellegren, H. & Möller, A.P. (1999) Breeding synchrony and paternity in the barn swallow (*Hirundo rustica*). *Behavioral Ecology and Sociobiology*, **45**, 211–218.
- Schwabl, H. (1996) Maternal testosterone in the avian egg enhances postnatal growth. *Comparative Biochemistry and Physiology. A, Comparative Physiology*, **114A**, 271–276.
- Schwabl, H. & Kriner, E. (1991) Territorial aggression and song of male European robins (*Erithacus rubecula*) in autumn and spring: effects of antiandrogen treatment. *Hormones and Behavior*, **25**, 180–194.
- Shizuka, D. & Dickinson, J.L. (2005) Using molt limits to age Western Bluebirds. *Journal of Field Ornithology*, **76**, 193–196.
- Sih, A., Bell, A. & Johnson, J.C. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, **19**, 372–378.
- Sockman, K.W., Schwabl, H. & Sharp, P.J. (2004) Removing the confound of time in investigating the regulation of serial behaviours: testosterone, prolactin and the transition from sexual to parental activity in male American kestrels. *Animal Behaviour*, **67**, 1151–1161.
- Sockman, K.W., Weiss, J., Webster, M.S., Talbott, V. & Schwabl, H. (2008) Sex-specific effects of yolk-androgens on growth of nestling American kestrels. *Behavioral Ecology & Sociobiology*, **62**, 617–625.
- Soma, K.K., Tramontin, A.D. & Wingfield, J.C. (2000) Oestrogen regulates male aggression in the non-breeding season. *Proceedings of the Royal Society of London. Series B*, **267**, 1089–1096.
- Strasser, R. & Schwabl, H. (2004) Yolk testosterone organizes behavior and male plumage coloration in house sparrows. *Behavioral Ecology*, **56**, 491–497.
- Stutchbury, B.J.M., Pitcher, T.E., Norris, D.R., Tuttle, E.M. & Gosner, R.A. (2005) Does male extra-territory foray effort affect fertilization success in hooded warblers *Wilsonia citrina*? *Journal of Avian Biology*, **36**, 471–477.
- Weatherhead, P.J. & Yezerinac, S.M. (1998) Breeding synchrony and extra-pair mating in birds. *Behavioral Ecology and Sociobiology*, **43**, 217–219.
- Williams, T.D., Ames, C.E., Kiparissis, Y. & Wynne-Edwards, K.E. (2005) Laying-sequence-specific variation in yolk oestrogen levels, and relationship to plasma oestrogen in female zebra finches (*Taeniopygia guttata*). *Proceedings of the Royal Society of London. Series B*, **272**, 173–177.
- Wingfield, J.C., Hegner, R.E., Alfred M. Dufty, J. & Ball, G.F. (1990) The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist*, **136**, 829–846.
- Wolf, M., van Doorn, G.S., Leimar, O. & Weissing, F.J. (2007) Life-history trade-offs favour the evolution of animal personalities. *Nature*, **447**, 581–585.

Received 27 April 2011; accepted 9 January 2012

Handling Editor: Jennifer Grindstaff