

Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged

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Keywords:

correlational selection;
GnRH challenge;
honest signals;
life-history trade-offs;
phenotypic integration;
plumage;
sexual selection;
testosterone.

Abstract

When male investment in mating varies with quality, reliable sexual signals may evolve. In many songbirds, testosterone mediates mating investment, suggesting that signals should be linked to testosterone production. However, because testosterone may change rapidly during behaviour such as territorial aggression and courtship, efforts to establish such a relationship have proved challenging. In a population of dark-eyed juncos, we measured individual variation in the production of short-term testosterone increases by injecting gonadotropin-releasing hormone (GnRH). We found a positive correlation between the magnitude of these increases and the size of a plumage ornament ('tail white') previously shown to be important for female choice and male-male competition. We then measured naturally elevated testosterone levels produced during male-male competition and found that they covaried with those induced by GnRH. We suggest that the association between tail white and testosterone increases may allow conspecifics to assess potential mates and competitors reliably using tail white.

Introduction

The origin and maintenance of reliability, or honesty, in sexual signals has been an issue of longstanding interest in evolutionary biology (Andersson, 1994; Kokko *et al.*, 2003; Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005). Reliable signals are expected to evolve when a male's optimal level of signalling depends on his quality, that is, under circumstances in which high-quality males achieve greater fitness with strong signals than do low-quality males (Nur & Hasson, 1984; Grafen, 1990; Michod & Hasson, 1990; Getty, 1998, 2006). Covariation between quality and signal expression can arise because mating success typically comes at the expense of other components of fitness such as survival, and higher-quality males are generally better able to afford investment in mating. The evolution of honest sexual signals is thus intimately linked to life-history trade-offs that involve allocation of time and energy to mating effort

vs. other fitness-related functions such as self-maintenance (Getty, 1998, 2006; Kokko, 1998; Kokko *et al.*, 2002).

The resolution of such life-history trade-offs is often proximately regulated by hormonal mechanisms (Stearns, 1992; Sinervo & Svensson, 1998; Ketterson & Nolan, 1999; Zera & Harshman, 2001; Ricklefs & Wikelski, 2002; Adkins-Regan, 2005). In songbirds, investment in mating effort is mediated in part by the steroid hormone testosterone. In many species, experimentally elevated testosterone tends to increase behaviours related to mating, such as song and display, while decreasing self-maintenance and parental care (Ketterson & Nolan, 1992, 1999; Adkins-Regan, 2005; Hau, 2007). Differential testosterone production therefore has the potential to act as a proximate mechanism allowing males to invest differentially in mating effort according to their quality. Thus, the predictions of signalling theory lead us to the expectation that variation in sexual signals, such as ornamental plumage, should often be correlated with natural variation in testosterone production.

Ornamental plumage is one of the most common types of sexual signal in songbirds (Hill, 2006; Senar, 2006).

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Although testosterone is generally not responsible for generating sexual dimorphism in plumage (as it often is for fleshy ornaments such as wattles and spurs; Owens & Short, 1995; Kimball, 2006), several recent studies have reported positive correlations between ornamental plumage and testosterone in male songbirds. The most well-supported case comes from house sparrows (*Passer domesticus*), in which testosterone appears to have a direct effect on the size of a male's bib (Evans *et al.*, 2000; Buchanan *et al.*, 2001; Gonzalez *et al.*, 2001; Strasser & Schwabl, 2004). Testosterone has been linked to plumage variation in other species as well. Circulating plasma testosterone has been linked to carotenoid colouration in the house finch (*Carpodacus mexicanus*; Duckworth *et al.*, 2004; also see Stoehr & Hill, 2001) and the structurally coloured crown of the blue tit (*Cyanistes caeruleus*; Peters *et al.*, 2006). Faecal testosterone was correlated with the size of the forehead patch of the collared flycatcher (*Ficedula albicollis*; Garamszegi *et al.*, 2004). Testosterone induces the prenuptial moult to breeding plumage in superb fairy-wrens (*Malurus cyaneus*; Peters *et al.*, 2000). Although these studies provide evidence for a possible role for testosterone in the evolution of honest plumage signals, all were focused on static as opposed to dynamic measurements of testosterone.

In nature, testosterone levels are not static but rather have the potential to vary greatly over time. Testosterone levels often show variation on long-term and short-term scales in response to seasonal changes and social stimuli respectively (Wingfield *et al.*, 1990). Transient, socially modulated elevations of testosterone are produced during behaviours associated with mate acquisition, namely male–male aggression and courtship of females (Harding, 1981; Wingfield, 1985; Wingfield *et al.*, 1990, 2001; Pinxten *et al.*, 2003; Goymann *et al.*, 2007). Thus, variation in short-term testosterone increases is likely to be closely linked to variation in the resolution of trade-offs involving mating effort (McGlothlin *et al.*, 2007a). Short-term modulation of testosterone is most evident in species that produce multiple broods each breeding season (Landys *et al.*, 2007). In these species, males may need to make rapid shifts between mating effort and parental effort because opportunities to obtain additional matings (via extra-pair copulations) overlap with the need to care for offspring. Because of the close association between the production of socially modulated testosterone levels and mating-related behaviour in such species, a relationship between plumage and transient testosterone changes would provide stronger evidence of the existence of links between ornamentation and allocation to mating effort than would a relationship with static testosterone levels.

In this study, we tested for a relationship between transient increases in testosterone and plumage in a free-living population of dark-eyed juncos (*Junco hyemalis*). A male's ability to produce temporary testosterone increases may be assessed by administering intramuscu-

lar injections of gonadotropin-releasing hormone ('GnRH challenges,' see Jawor *et al.*, 2006). *In vivo*, GnRH is released from the hypothalamus, stimulating the release of luteinizing hormone from the pituitary, which in turn temporarily increases production and release of testosterone from the gonads. In juncos, the testosterone response to GnRH challenges shows repeatable variation among individuals across the breeding season (Jawor *et al.*, 2006). Further, natural variation in transient testosterone elevations has been linked to natural variation in trade-off between mating effort and parental effort (McGlothlin *et al.*, 2007a). Specifically, maximum testosterone levels following GnRH challenges accurately predicted increased aggression in response to a territorial intruder, a measure of mating effort, whereas the magnitude of the GnRH-induced increase in testosterone predicted decreased nestling feeding, a measure of parental effort (McGlothlin *et al.*, 2007a). To our knowledge, only one other study has tested for a relationship between plumage and short-term testosterone increases. Spinney *et al.* (2006) found that alternative plumage morphs of captive white-throated sparrows (*Zonotrichia albicollis*), which differ in aggressiveness and reproductive strategy, also differ in testosterone levels produced in response to GnRH challenges.

We measured the phenotypic correlation between testosterone increases produced in response to a GnRH challenge (potential androgen response, *sensu* Goymann *et al.*, 2007) and an attractive plumage ornament (a white patch on the tail, or 'tail white,' Hill *et al.*, 1999; Wolf *et al.*, 2004; McGlothlin *et al.*, 2005). Male juncos use tail white in both courtship and aggressive displays, and males with experimentally enhanced tail white become socially dominant and are more attractive to females (Balph *et al.*, 1979; Holberton *et al.*, 1989; Hill *et al.*, 1999; Fig. 1). In nature, tail white and body size have been found to be under correlational sexual selection, such that coordinated expression of tail white and body size leads to greater mating success than mismatches of the two traits (McGlothlin *et al.*, 2005). If tail white serves as an honest signal of male quality, we would expect males with more white to invest more heavily in mating effort via increased testosterone production. We, therefore, predicted that males with whiter tails would respond more strongly to GnRH.

To validate that the testosterone response to a GnRH injection reflects a male's actual tendency to produce testosterone in the wild (male–male androgen response, *sensu* Goymann *et al.*, 2007), we correlated GnRH-challenge response with testosterone produced after simulated territorial intrusions (STIs). We predicted that males that responded more strongly to GnRH would also generate larger increases in testosterone after an intrusion. If our predictions were borne out, we would conclude that males earlier shown to be more attractive, i.e. those with whiter tails, also possess the physiology known to accompany more intense mate-acquisition



Fig. 1 Tail spreading behaviour of a male dark-eyed junco. During courtship, males erect their body plumage and spread their tail to reveal the white patch on their outer tail feathers, which is mostly hidden at rest. Testosterone increases the frequency of this display, and increasing the size of the patch increases its attractiveness to females. Males also display their tails during intrasexual interactions, and males with more tail white are socially dominant. Photograph taken from video footage by Britt Heidinger.

behaviour. This covariation between signal, physiology and behaviour would suggest that potential competitors and mates should be able to predict the outcome of interactions with an individual by assessing his ornamentation.

Materials and methods

Study area and species

We studied a resident population of the Carolina subspecies of the dark eyed junco (*J. h. carolinensis*) that breeds at and around Mountain Lake Biological Station (MLBS) in Giles County, VA, USA (37°22'N, 80°32'W). Males defend breeding territories upon which a single female nests. Both parents care for offspring, but mating often occurs outside the pair, generating opportunity for sexual selection among males (Ketterson *et al.*, 1997; Nolan *et al.*, 2002; McGlothlin *et al.*, 2005).

Capture

In April–August 2003–2004, males ($n = 90$) were captured using mist nets or Potter traps. Upon capture, birds were transported to a central laboratory at MLBS in a holding bag. If previously uncaptured, birds were given a numbered aluminium leg band and a unique combination of plastic colour leg bands for identification. We determined age (yearling or older adult [≥ 2 years]) by examining the colour of the primary wing coverts, and secondarily, the iris, which are both lighter in yearlings

(Nolan *et al.*, 2002). Mass (g) was measured using a spring balance.

GnRH challenges

Each time a bird was captured, a blood sample was obtained from the wing vein (initial sample). Handling time was recorded as the time in minutes from capture to collection of this blood sample, averaging 48 min (range 2–217 min; Jawor *et al.*, 2006). A solution of 1.25 μg chicken GnRH-I (Sigma L0637; St Louis, MO, USA) in 50 μL of 0.1 M phosphate-buffered saline was then injected into the pectoral muscle. The bird was returned to its holding bag, and after exactly 30 min, a second blood sample was collected (post-challenge sample). After this sample, the bird was released at the site of capture. Plasma was separated and frozen ($-20\text{ }^{\circ}\text{C}$) for later hormone analysis.

To control for the idiosyncrasies of capture and to obtain robust individual estimates of testosterone production, we attempted to obtain four samples each year from individual birds, collected at four sampling stages across the breeding season (Jawor *et al.*, 2006). We attempted to obtain two samples during early breeding (21 April–16 May) by catching birds at random in baited mist nets and traps. The first GnRH challenge was administered upon each bird's first capture (2003: 28 April–16 May, $n = 53$; 2004: 21 April–11 May, $n = 44$, combined $n = 97$) and the second after waiting 7–21 days (mean 10.4; 2003: 6 May–16 May, $n = 26$; 2004: 1 May–15 May, $n = 11$, combined $n = 37$). During early breeding, many birds were beginning to nest, but the exact stage of reproduction was unknown for most of them (dates of first egg were 26 April in 2003 and 25 April in 2004). Some birds were captured and given a GnRH challenge while feeding 6–7-day-old nestlings (2003: 25 May–29 June, $n = 14$, 2004: 20 May–20 July, $n = 14$, combined $n = 28$). Captures during this stage were made by placing a mist net at the nest. A final set of birds was captured at the end of the breeding season, but prior to the onset of moult, using baited mist nets (2003: 15 July–6 August, $n = 7$; 2004: 20 July–5 August, $n = 9$). All sampling periods occurred after the typical early-breeding season testosterone peak (26 March–14 April; Ketterson & Nolan, 1992). Overall, five individuals were challenged a total of five times, five were challenged four times, 12 were challenged thrice, 29 were challenged twice and 39 were challenged once. Twenty-one individuals received challenges in both 2003 and 2004, 37 were challenged in 2003 only and 32 were challenged in 2004 only. Thirty-two males were sampled only as yearlings, 54 were sampled only as older adults and 4 were sampled in 2003 as yearlings and in 2004 as older adults.

Our GnRH-challenge method stimulates a maximal testosterone response at 30 min, and levels return to baseline within 2 h (Jawor *et al.*, 2006). In our

population, there are significant differences among the sampling periods described earlier in the increase of testosterone produced, indicating a gradual seasonal decline (Jawor *et al.*, 2006; see also section 'Results'). When seasonal variation is held constant, individuals show repeatable differences in the magnitude of testosterone increases above initial levels (repeatability = 0.36) (Jawor *et al.*, 2006).

Tail white measurement

To assess ornament size, digital photographs of individual tail feathers were obtained upon first capture of males in 2003 and 2004 ($n = 90$). Photographs were taken of the three to five feathers on the right side of the tail that have some degree of white colouration by placing a piece of black paper between the tail feathers (Wolf *et al.*, 2004; Yeh, 2004). If a feather on the right was missing, the feather on the left was photographed in its place. So that the entire feather could be seen, body feathers were held to the side with the handler's thumb. Photographs were taken from a standardized distance, using standardized lighting, and with a ruler for scale. Using the MetaView/MetaMorph image analysis program (Universal Imaging, Sunnyvale, CA, USA), the proportion of white area of each feather on the right side of the tail was measured. This proportion, between 0 and 1, was the tail white value for each feather. To obtain an individual's tail white score, the values of the feathers on the right side of the tail were added (range 1.80–3.26; Wolf *et al.*, 2004; McGlothlin *et al.*, 2005).

Simulated territorial intrusions

In order to validate that GnRH-induced testosterone levels predict those naturally produced in response to social stimuli, we performed both GnRH challenges and STIs on a small sample of ten males in May–July 2005. GnRH challenges were performed on males captured using mist nets at their nest while feeding 6-day-old offspring. STIs were conducted 2–4 days later, after the nestlings had been collected (as part of a separate experiment) or the nest had been naturally depredated. All STIs were started before 09:00. In each STI, we placed a caged captive male in the territory near the empty nest and played a 10-min CD recording of junco songs, which were recorded in our population >10 years before. Songs were played at a rate of four songs per minute. This treatment stimulated approach and directed song by the territorial male. After 10 min, the recording was stopped, at which time two mist nets near the cage were unfurled in an attempt to capture the focal male. After 10 min of silence, the playback was restarted and remained on until the male was captured. A blood sample was taken immediately and plasma was reserved for hormone analysis. Captures occurred 13–86 min after the playback was restarted (mean 37.1 min).

Testosterone assays

Testosterone levels were determined using a commercial enzyme immunoassay (Assay Designs #901-065; Ann Arbor, MI, USA). Assay methods are described in detail elsewhere (Clotfelter *et al.*, 2004). Approximately 2000 cpm of tritiated testosterone were added to each sample in order to calculate recoveries after extraction ($2 \times$ diethyl ether). Extracts were resuspended in 50- μ L ethanol and diluted to 350 μ L with assay buffer from the kit. From each reconstituted sample, 100 μ L were used to determine recoveries, and duplicate 100- μ L quantities were used in the EIA. Testosterone concentrations were determined with a 4-parameter logistic curve-fitting program (Microplate Manager; BioRad Laboratories, Inc., Hercules, CA, USA) and corrected for incomplete recoveries.

Samples from different years were run in different assays. In 2003–2004, intraplate coefficients of variation ranged from 1 to 19% (mean 9%), and interplate variation was 20%. In 2005, intraplate variation was 3–4% (mean 3%), and interplate variation was 8%. Within each dataset, we corrected for interplate variation by multiplying each measurement by the grand mean of assay standards across all plates within the dataset and dividing by the plate mean of these standards.

Statistical analyses

To test for a relationship between testosterone production and tail white, it was necessary to correct for factors that may have influenced the response to GnRH (such as season; Jawor *et al.*, 2006) as well as the nonindependence of data points caused by repeated sampling. To this end, we used restricted maximum likelihood to fit linear mixed models. Mixed models allow tests of multiple fixed effects while allowing for structured random effects (Verbeke & Molenberghs, 2000). In our models, the random portion accounted for repeated measures taken from a single individual as well as the structure of our sampling regime (eight total sampling periods, i.e. four seasonal stages in each of 2 years). Our model estimated the error variance–covariance matrix of the data with a first-order factor analytic structure (SPSS 14.0 Command Syntax Reference, SPSS Inc., Chicago, IL, USA). The diagonal elements ($\lambda_i^2 + d$) of this matrix were estimates of the error variance at each sampling period, and the off-diagonals (λ_i, λ_j) were estimates of the within individual covariance between sampling periods. Thus, 36 error (co)variance components were described by nine model parameters (eight λ and one d). This covariance matrix was similar to that generated by a model that estimated all 36 components separately, but allowed for greater power because of the reduction in the number of parameters.

In the fixed portion of the models, we included tail white as a continuous predictor. We also included other

variables that might explain variation in measures of testosterone. As categorical predictors, we included year, seasonal stage (early breeding A and B, nestling feeding, or late breeding) and a year–stage interaction; we included mass (g) and handling time (min, ln-transformed) as covariates (see Jawor *et al.*, 2006 for discussion of these variables). We used type I (sequential) sums of squares, which allowed us to control for these effects before testing for covariation with tail white, which was entered into the model last.

As dependent variables, we used initial and post-GnRH challenge testosterone (both ln-transformed) as well as the GnRH-induced increase in testosterone (ln post-challenge–ln initial). In the present study, post-challenge testosterone was positively correlated with initial testosterone ($r_{176} = 0.54$, $P < 0.0001$) and GnRH-induced increase ($r_{176} = 0.82$, $P < 0.0001$), but initial testosterone was not significantly correlated with GnRH-induced increase ($r_{176} = -0.04$, $P < 0.60$). Despite this pattern of correlation, these three measures show different relationships with male behaviour (McGlothlin *et al.*, 2007a); thus, we analyze them separately here.

Because mating strategies and plumage-hormone relationships may differ between first-year breeders and older birds (e.g. Peters *et al.*, 2006), we performed a further test to determine whether age classes differed in our population. To the model described earlier, we added age class as a main effect and nested tail white within age class. This type of model estimates age-specific slopes, and is equivalent to including an interaction term in an analysis of covariance (Engqvist, 2005). We performed this test only for GnRH-induced increases, which showed the strongest relationship with tail white (see section 'Results').

Because STI data were only collected at one seasonal stage and were not repeated within individuals, we used standard parametric statistics (paired *t*-test and Pearson correlation) on ln-transformed testosterone values. All analyses were performed using SPSS 14 for Windows.

Results

We found a significant positive correlation between tail white and the magnitude of GnRH-induced testosterone increases (Table 1, Fig. 2). This relationship held even when we did not control for other fixed effects ($b = 0.26$, $F_{1,137.8} = 6.82$, $P = 0.010$). The relationships between tail white and both initial and post-challenge testosterone levels were nonsignificant (Table 1).

For each testosterone measure, there were effects of year and/or seasonal stage (Table 1). As in Jawor *et al.* (2006), post-challenge testosterone and GnRH-induced increases were weaker in later seasonal stages (Table 1). Mass affected response to GnRH challenges, with heavier birds showing lower post-challenge testosterone and GnRH-induced testosterone increase (Table 1, models 2 and 3). Additionally, handling time had significant

Table 1 Linear mixed models of testosterone before and after GnRH challenges.

Fixed effects	<i>F</i>	d.f.	<i>b</i>	<i>P</i>
ln Initial testosterone				
Year	11.00	1, 60.7		0.002
Stage	1.64	3, 47.5		0.19
Year × stage	0.95	3, 41.4		0.43
Mass	1.07	1, 144.5	0.002	0.30
ln handling time	7.70	1, 136.1	-0.09	0.006
Tail white	2.19	1, 139.7	-0.10	0.14
ln post-challenge testosterone				
Year	7.28	1, 83.0		0.008
Stage	18.82	3, 54.2		<0.001
Year × stage	2.68	3, 42.7		0.059
Mass	14.21	1, 116.2	-0.10	<0.001
ln handling time	5.68	1, 114.8	-0.10	0.019
Tail white	1.24	1, 119.0	0.14	0.27
GnRH-induced increase (ln post-challenge–ln initial)				
Year	3.25	1, 96.5		0.075
Stage	22.06	3, 73.1		<0.001
Year × stage	2.17	3, 65.5		0.101
Mass	27.81	1, 115.0	-0.10	<0.001
ln handling time	4.65	1, 101.3	-0.07	0.033
Tail white	5.17	1, 125.8	0.23	0.025

Fixed effects with $P < 0.05$ are highlighted in bold, effects with $0.10 < P < 0.05$ are shown in italics. Random effects (not shown) described and corrected for the pattern of residual (co)variance that arose from repeated sampling from individuals. See section 'Materials and methods' for details of the models.

negative effects on all measurements of testosterone (Table 1).

When we considered age classes of males separately, there was no significant main effect of age class on GnRH-induced testosterone increases ($F_{1,130.5} = 1.10$, $P = 0.30$), but the effect of tail white significantly differed between age classes ($F_{1,101.2} = 5.50$, $P = 0.005$). Specifically, the slope for first-year adults was strong and positive ($b = 0.48$, $t_{82.4} = 3.31$, $P = 0.001$), whereas there was no significant relationship in older adults ($b = 0.05$, $t_{130.3} = 0.32$, $P = 0.75$; see Fig. 2).

Territorial males presented with STIs displayed significantly elevated testosterone levels following the intrusion (paired *t*-test, $t_9 = 9.82$, $P < 0.0001$; Fig. 3a). Further, the levels produced during STIs were highly correlated with those produced by GnRH challenges ($r_8 = 0.68$, $P = 0.03$; Fig. 3b).

Discussion

In this study, we found that free-living male juncos with naturally larger tail white ornaments, a plumage trait associated with attractiveness and dominance, produced larger short-term increases in testosterone in response to injections of the hypothalamic hormone GnRH. Further, testosterone levels produced in response to GnRH were highly correlated with those produced after a STI in the

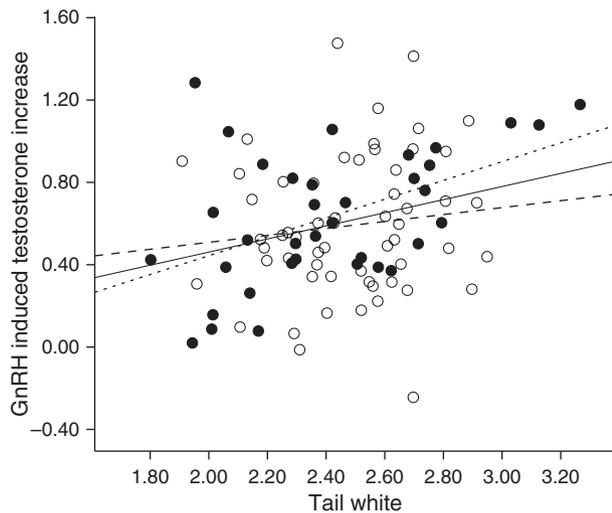


Fig. 2 Age-specific relationship between tail white and magnitude of testosterone increase after GnRH challenge (\ln post-challenge testosterone $-\ln$ initial testosterone). First-year males are shown as filled circles, and older adults are shown as open circles. Data points represent individual means. Males that were measured only as yearlings or older adults (86) are represented by a single data point, and four males that were measured in both age classes are represented by two points. Testosterone measures were adjusted for multiple measurements, as well as stage, mass and handling time, by using general linear models to calculate individual least-square means. This was performed separately for each age class. The solid line represents the line of best fit for the entire population, the dotted line for yearlings and the dashed line for older adults.

field, thus validating that GnRH injections may be used to predict natural hormonal responses. Overall, these results suggest that males with more attractive ornaments have the ability to produce larger short-term testosterone increases in response to social stimuli. To our knowledge, our study is the first to associate such testosterone increases with variation in a sexual signal in a natural population. This association may provide a physiological link between plumage and expression of behaviour, thus playing a role in maintaining the reliability of tail white as a signal.

Honest signalling

Testosterone is associated with the expression of a large suite of behaviours in male songbirds (Ketterson & Nolan, 1999; Adkins-Regan, 2005), and thus the correlation between tail white and testosterone production reported here suggests that many of a male's behavioural tendencies may be predicted from his plumage. One type of information that tail white may convey is a male's readiness to defend his territory, as territorial aggression has been intimately associated with transient testosterone increases in a number of species (e.g. Wingfield *et al.*, 1987; but see Landys *et al.*, 2007). In a related study of

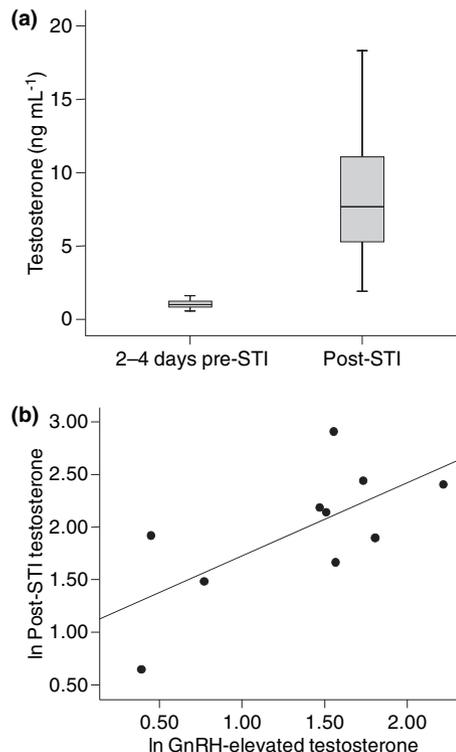


Fig. 3 Testosterone in response to a simulated territorial intrusion. (a) Testosterone shows a significant increase following a simulated territorial intrusion. The line represents the median, the shaded box represents the interquartile range and the bars represent the range. (b) Magnitude of the hormonal response to the intrusion is predicted by the response to a GnRH challenge. Line of best fit from a least-squares regression is presented for visualization.

juncos, we found that the intensity of territorial aggression was positively correlated with maximum levels of testosterone produced in response to GnRH challenges (McGlothlin *et al.*, 2007a).

Tail white is assessed by females during mate choice (Hill *et al.*, 1999), so the correlation between tail white and testosterone production should make tail white informative in this context as well. Both experimentally elevated testosterone and higher GnRH-induced testosterone increases are associated with decreased parental care in male juncos (Ketterson *et al.*, 1992; Cawthorn *et al.*, 1998; Schoech *et al.*, 1998; McGlothlin *et al.*, 2007a). Females that mate with males with larger tail white ornaments may thus receive little parental assistance from their social partners. It is unclear how mating with such males affects the fitness of the female and her offspring. Theory predicts that in species in which females prefer to mate with males that provide little parental assistance, females should receive indirect (i.e. genetic) benefits from such males (Kokko, 1998). Although testosterone production has not been directly linked to male genetic quality (i.e. the breeding value for

total fitness; Hunt *et al.*, 2004), such a relationship is suggested by the significant costs associated with elevated testosterone (Folstad & Karter, 1992; Buchanan *et al.*, 2001; Wingfield *et al.*, 2001; Roberts *et al.*, 2004; Reed *et al.*, 2006). Alternatively, males that readily defend their territories may provide females with direct benefits other than transporting food to offspring, such as high-quality territories.

Although its correlation with testosterone production appears to make tail white an informative signal, this relationship must be evolutionarily stable in order for signal honesty to be maintained. There are several possible explanations, both proximate and ultimate, for how this may occur. First, both testosterone and tail white may be condition-dependent. However, this possibility is unlikely because of the different time scales on which tail white and testosterone are expressed (Hill *et al.*, 1999). Furthermore, white plumage is generally thought to be inexpensive to produce (Prum, 2006), although tail white is weakly affected by diet quality (McGlothlin *et al.*, 2007b). Another possible proximate mechanism is a direct physiological link between testosterone and the expression of tail white, as is the case with the house sparrow's bib (e.g. Evans *et al.*, 2000). This is also unlikely, as an adult male's tail white may change only during the annual moult, a time when testosterone levels are extremely low and experimentally elevated testosterone inhibits moult (Nolan *et al.*, 1992; high doses, and J. M. Jawor, J. W. McGlothlin, and E. D. Ketterson, unpublished data, low doses) However, this does not preclude an early developmental effect of testosterone (Strasser & Schwabl, 2004). On an ultimate level, the correlation between tail white and testosterone production may be maintained by correlational selection if they interact to affect fitness (Cheverud, 1982; Lande & Arnold, 1983; Phillips & Arnold, 1989; Brodie, 1992; Sinervo & Svensson, 2002). Although the pattern of selection in the wild is unknown, the two traits do exhibit functional interactions that make correlational selection a possibility. Both are important for male-male competition, where males may have to reinforce their tail white signal with aggressive behaviour (Holberton *et al.*, 1989; Senar, 2006; McGlothlin *et al.*, 2007a). When courting females, tail white and testosterone are likely to interact to produce an attractive display (Fig. 1) (Enstrom *et al.*, 1997; Hill *et al.*, 1999).

Age-dependent effects

Although older males tend to have more tail white (Wolf *et al.*, 2004; Yeh, 2004; McGlothlin *et al.*, 2005), the change in testosterone levels in response to GnRH was not significantly related to age (see Jawor *et al.*, 2006 for further discussion). We found, however, that the strength of the correlation between tail white and testosterone production changed with age. Specifically, the relationship was strongly positive in first-year adults,

but not significantly different from zero in older adults. We stress that this finding does not negate the correlation between tail white and testosterone increases at the population level. Juncos should still be able to predict the likely behaviour of an unfamiliar male, irrespective of age, based on the size of his tail white patch. Thus, because a population consists of a mixture of old and young adults, tail white should remain 'honest on average' (Kokko, 1997). However, the extent to which tail white is informative may depend on the age structure of the population.

The relationship between plumage and testosterone may be weaker in older males because selection pressures differ for the two age classes. One possibility is that correlational selection may act more strongly on younger birds, favouring a stronger relationship between testosterone production and tail white, whereas such selection is relaxed for older adults. This may occur because the decision of whether to divert energy away from mating effort and towards parental effort is more crucial for younger birds. Older male juncos seem to have a general mating advantage; they often retain territories and mates from previous years (Nolan *et al.*, 2002) and are more likely than yearlings to obtain extra-pair mates, suggesting that they may be more attractive to females in general (Reed *et al.*, 2006). Because of competition from older males, it may be beneficial for first-year males with smaller ornaments to invest more in their own survival with the expectation of obtaining higher mating success as an older adult. However, first-year males with large ornaments may be able to succeed in competition with older males, thus favouring a heavier investment in mating.

Another, not mutually exclusive, explanation for why older males show a decreased correlation is that they have already experienced more episodes of survival selection. Stabilizing selection acting on tail white, testosterone production or both could eliminate extremes from the population, weakening the correlation between the variables in older cohorts.

Interestingly, Peters *et al.* (2006) showed a similar pattern in blue tits. In that species, old and young males show opposite relationships between plumage and testosterone corresponding to opposite relationships between plumage and mating success. The results of that study, combined with those presented here, suggest that future work should focus on how selection shapes the age-dependence of mating strategies and the expression of sexually selected traits.

Conclusion

In summary, our results demonstrate a correlation between static, sexually selected morphology and a dynamic physiological mechanism known to mediate allocation to mating effort. This relationship may account for the honesty of tail white in juncos, and we predict

that similar relationships are likely to occur in other species, particularly when the trade-off between mating effort and parental effort is important. In biparental songbirds that exhibit social modulation of testosterone, we predict that sexual ornaments are more likely to be associated with the ability to produce large changes in testosterone levels rather than high circulating levels (see also Spinney *et al.*, 2006). This may explain why few studies have found relationships between plumage and testosterone (Owens & Short, 1995; Kimball, 2006).

Together with previous studies in this species (Enstrom *et al.*, 1997; Raouf *et al.*, 1997; Hill *et al.*, 1999; Ketterson & Nolan, 1999; Ketterson *et al.*, 2001; McGlothlin *et al.*, 2005; Reed *et al.*, 2006), our results provide links among hormone levels, ornamental traits and behaviour at the level of individual variation, the raw material on which selection can act. Because of the roles of testosterone in mediating mating effort and display, the full potential benefits of a large tail white patch are likely to be realized only if it is associated with high levels of testosterone production. Thus, correlational selection may be important for maintaining the correlation between testosterone production and tail white, and hence, signal reliability (Getty, 1998, 2006; McGlothlin *et al.*, 2005). This form of selection has been shown to act on tail white and body size in our population (McGlothlin *et al.*, 2005). However, despite extensive information on the fitness effects of experimentally elevated testosterone (Reed *et al.*, 2006), we know little about how natural variation in testosterone is related to fitness in the wild. Future studies should examine how selection shapes individual variation in sexual morphology, physiology and behaviour, and the relationships among them.

Acknowledgments

We thank J. Gaudioso, N. Gerlach, A. Lindsay, D. O'Neal, K. Pavlis, S. Schrock, E. Snajdr, P. Stevens, C. Ziegenfus and D. Zysling for assistance in the field; L. Engqvist and E. Martins for statistical advice and E. Brodie III, G. Ball, G. Demas, B. Heidinger, M. Maas, T. Price, V. Rudolf, S. Sakaluk and D. Sengelaub for discussions and comments on the manuscript, and Mountain Lake Biological Station (H. Wilbur, Director and E. Nagy, Associate Director) and Mountain Lake Hotel for facilities and permission to work on their land. This research was supported by a National Science Foundation Doctoral Dissertation Improvement Grant (0508693) and grants from the American Ornithologists' Union, Indiana University, Mountain Lake Biological Station, Sigma Xi and the Wilson Ornithological Society to JWM, a National Science Foundation Research Experience for Undergraduates award to JLP and a National Science Foundation grant (0216091) to EDK. This research adhered to the Association for the Study of Animal Behaviour/Animal Behavior Society Guidelines for the Use of Animals in Research, the legal requirements of

the United States of America, the State of Indiana, and the Commonwealth of Virginia, and the guidelines of the Animal Care and Use Committees at Indiana University and the University of Virginia.

References

- Adkins-Regan, E. 2005. *Hormones and Animal Social Behavior*. Princeton University Press, Princeton.
- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.
- Balgh, M.H., Balgh, D.F. & Romesburg, C. 1979. Social status signaling in winter flocking birds: an examination of current hypotheses. *Auk* **96**: 78–93.
- Brodie, E.D. III 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution* **46**: 1284–1298.
- Buchanan, K.L., Evans, M.R., Goldsmith, A.R., Bryant, D.M. & Rowe, L.V. 2001. Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc. R. Soc. Lond. B* **268**: 1337–1344.
- Cawthorn, J.M., Morris, D.L., Ketterson, E.D. & Nolan, V. Jr 1998. Influence of experimentally elevated testosterone on nest defence in dark-eyed juncos. *Anim. Behav.* **56**: 617–621.
- Cheverud, J.M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution* **36**: 499–516.
- Clotfelter, E.D., O'Neal, D.M., Gaudioso, J.M., Casto, J.M., Parker-Renga, I.M., Snajdr, E.A., Duffy, D.L., Nolan, V. Jr & Ketterson, E.D. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Horm. Behav.* **46**: 171–178.
- Duckworth, R.A., Mendonça, M.T. & Hill, G.E. 2004. Condition-dependent sexual traits and social dominance in the house finch. *Behav. Ecol.* **15**: 779–784.
- Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim. Behav.* **70**: 967–971.
- Enstrom, D.A., Ketterson, E.D. & Nolan, V. Jr 1997. Testosterone and mate choice in the dark-eyed junco. *Anim. Behav.* **54**: 1135–1146.
- Evans, M.R., Goldsmith, A.R. & Norris, S.R.A. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **47**: 156–163.
- Folstad, I. & Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**: 603–622.
- Garamszegi, L.Z., Møller, A.P., Torok, J., Michl, G., Peczely, P. & Richard, M. 2004. Immune challenge mediates vocal communication in a passerine bird: an experiment. *Behav. Ecol.* **15**: 148–157.
- Getty, T. 1998. Handicap signalling: when fecundity and viability do not add up. *Anim. Behav.* **56**: 127–130.
- Getty, T. 2006. Sexually selected signals are not similar to sports handicaps. *Trends Ecol. Evol.* **21**: 83–88.
- Gonzalez, G., Sorci, G., Smith, L.C. & de Lope, F. 2001. Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **50**: 557–562.
- Goymann, W., Landys, M.M. & Wingfield, J.C. 2007. Distinguishing seasonal androgen responses from male–male andro-

- gen responsiveness – revisiting the challenge hypothesis. *Horm. Behav.* **51**: 463–476.
- Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* **144**: 517–546.
- Harding, C.F. 1981. Social modulation of circulating hormone levels in the male. *Am. Zool.* **21**: 223–231.
- Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* **29**: 133–144.
- Hill, G.E. 2006. Female mate choice for ornamental coloration. In: *Bird Coloration*, Vol. 2 (G. E. Hill & K. J. McGraw, eds), pp. 137–200. Harvard University Press, Cambridge, MA.
- Hill, J.A., Enstrom, D.A., Ketterson, E.D., Nolan, V. Jr & Ziegenfuss, C. 1999. Mate choice based on static versus dynamic secondary sexual traits in the dark-eyed junco. *Behav. Ecol.* **10**: 91–96.
- Holberton, R.L., Able, K.P. & Wingfield, J.C. 1989. Status signaling in dark-eyed juncos, *Junco hyemalis*: plumage manipulations and hormonal correlates of dominance. *Anim. Behav.* **37**: 681–689.
- Hunt, J., Bussière, L.F., Jennions, M. D. & Brooks, R. 2004. What is genetic quality? *Trends Ecol. Evol.* **19**: 329–333.
- Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E. & Ketterson, E.D. 2006. Seasonal and individual variation in response to GnRH challenge in male dark-eyed juncos (*Junco hyemalis*). *Gen. Comp. Endo.* **149**: 182–189.
- Ketterson, E.D. & Nolan, V. Jr 1992. Hormones and life histories: an integrative approach. *Am. Nat.* **140**: S33–S62.
- Ketterson, E.D. & Nolan, V. Jr 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* **154**: S4–S25.
- Ketterson, E.D., Nolan, V. Jr, Wolf, L. & Ziegenfuss, C. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *Am. Nat.* **140**: 980–999.
- Ketterson, E.D., Parker, P.G., Raouf, S.A., Nolan, V. Jr, Ziegenfuss, C. & Chandler, C.R. 1997. The relative impact of extra-pair fertilizations on variation in male and female reproductive success in dark-eyed juncos (*Junco hyemalis*). In: *Avian Reproductive Tactics; Female and Male Perspectives* (P. G. Parker & N. T. Burley, eds), pp. 81–101. Ornithological Monograph no. 49. Allen Press, Lawrence, KS.
- Ketterson, E.D., Nolan, V. Jr, Casto, J.M., Buerkle, C.A., Clotfelter, E., Grindstaff, J.L., Jones, K.J., Lipar, J.L., McNabb, F.M.A., Neudorf, D.L., Parker-Renga, I., Schoech, S.J. & Snajdr, E. 2001. Testosterone, phenotype, and fitness: a research program in evolutionary behavioral endocrinology. In: *Avian Endocrinology* (A. Dawson & C. M. Chaturvedi, eds), pp. 19–40. Narosa Publishing House, New Delhi, India.
- Kimball, R.T. 2006. Hormonal control of coloration. In: *Bird Coloration*, Vol. 1 (G. E. Hill & K. J. McGraw, eds), pp. 431–468. Harvard University Press, Cambridge, MA.
- Kokko, H. 1997. Evolutionary stable strategies of age-dependent sexual advertisement. *Behav. Ecol. Sociobiol.* **41**: 99–107.
- Kokko, H. 1998. Should advertising parental care be honest? *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 1871–1878.
- Kokko, H., Brooks, R., McNamara, J.M. & Houston, A.I. 2002. The sexual selection continuum. *Proc. R. Soc. Lond. B Biol. Sci.* **269**: 1331–1340.
- Kokko, H., Brooks, R., Jennions, M.D. & Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 653–664.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Landys, M.M., Goymann, W., Raess, M. & Slagsvold, T. 2007. Hormonal responses to male–male social challenge in the blue tit *Cyanistes caeruleus*: single-broodedness as an explanatory variable. *Physiol. Biochem. Zool.* **80**: 228–240.
- Maynard Smith, J. & Harper, D. 2003. *Animal Signals*. Oxford University Press, Oxford.
- McGlothlin, J.W., Parker, P.G., Nolan, V. Jr & Ketterson, E.D. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. *Evolution* **59**: 658–671.
- McGlothlin, J.W., Jawor, J.M. & Ketterson, E.D. 2007a. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *Am. Nat.* **170**: 864–875.
- McGlothlin, J.W., Duffy, D.L., Henry-Freeman, J.L. & Ketterson, E.D. 2007b. Diet quality affects an attractive white plumage pattern in dark-eyed juncos (*Junco hyemalis*). *Behav. Ecol. Sociobiol.* **61**: 1391–1399.
- Michod, R.E. & Hasson, O. 1990. On the evolution of reliable indicators of fitness. *Am. Nat.* **135**: 788–808.
- Nolan, V. Jr, Ketterson, E.D., Ziegenfuss, C., Cullen, D.P. & Chandler, C.R. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on prebasic molt and survival in male Dark-eyed Juncos. *Condor* **94**: 364–370.
- Nolan, V. Jr, Ketterson, E.D., Cristol, D.A., Rogers, C.M., Clotfelter, E.D., Titus, R.C., Schoech, S.J. & Snajdr, E. 2002. Dark-eyed junco (*Junco hyemalis*). In: *The Birds of North America*, No. 716 (A. Poole & F. Gill, eds), pp. 1–44. The Birds of North America, Inc., Philadelphia, PA.
- Nur, N. & Hasson, O. 1984. Phenotypic plasticity and the handicap principle. *J. Theor. Biol.* **110**: 275–297.
- Owens, I.P.F. & Short, R.V. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends Ecol. Evol.* **10**: 44–47.
- 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*. *Behav. Ecol. Sociobiol.* **47**: 438–445.
- Peters, A., Delhey, K., Goymann, W. & Kempenaers, B. 2006. Age-dependent association between testosterone and crown UV coloration in male blue tits (*Parus caeruleus*). *Behav. Ecol. Sociobiol.* **59**: 666–673.
- Phillips, P.C. & Arnold, S.J. 1989. Visualizing multivariate selection. *Evolution* **43**: 1209–1222.
- Pinxten, R., De Ridder, E. & Eens, M. 2003. Female presence affects male behavior and testosterone levels in the European starling (*Sturnus vulgaris*). *Horm. Behav.* **44**: 103–109.
- Prum, R.O. 2006. Anatomy, physics, and evolution of structural colors. In: *Bird Coloration*, Vol. 1 (G. E. Hill & K. J. McGraw, eds), pp. 295–353. Harvard University Press, Cambridge.
- Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan, V. Jr & Ziegenfuss, C. 1997. Testosterone affects reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos (*Junco hyemalis*). *Proc. R. Soc. Lond. B* **264**: 1599–1603.
- Reed, W.L., Clark, M.E., Parker, P.G., Raouf, S.A., Arguedas, N., Monk, D.S., Snajdr, E., Nolan, V. Jr & Ketterson, E.D. 2006. Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. *Am. Nat.* **167**: 667–683.

- Ricklefs, R.E. & Wikelski, M. 2002. The physiology/life-history nexus. *Trends Ecol. Evol.* **17**: 462–467.
- Roberts, M.L., Buchanan, K.L. & Evans, M.R. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.* **68**: 227–239.
- Schoech, S.J., Ketterson, E.D., Nolan, V. Jr, Sharp, P.J. & Buntin, J.D. 1998. The effect of exogenous testosterone on parental behavior, plasma prolactin, and prolactin binding sites in dark-eyed juncos. *Horm. Behav.* **34**: 1–10.
- Searcy, W.A. & Nowicki, S. 2005. *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton University Press, Princeton.
- Senar, J.C. 2006. Color displays as intrasexual signals of aggression and dominance. In: *Bird Coloration*, Vol. 2 (G. E. Hill & K. J. McGraw, eds), pp. 87–136. Harvard University Press, Cambridge.
- Sinervo, B. & Svensson, E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* **83**: 432–442.
- Sinervo, B. & Svensson, E. 2002. Correlational selection and the evolution of genomic architecture. *Heredity* **89**: 329–338.
- Spinney, L.H., Bentley, G.E. & Hau, M. 2006. Endocrine correlates of alternative phenotypes in the white-throated sparrow (*Zonotrichia albicollis*). *Horm. Behav.* **50**: 762–771.
- Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, New York.
- Stoehr, A.M. & Hill, G.E. 2001. The effects of elevated testosterone on plumage hue in male House Finches. *J. Avian Biol.* **32**: 153–158.
- Strasser, R. & Schwabl, H. 2004. Yolk testosterone organizes behavior and male plumage coloration in house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **56**: 491–497.
- Verbeke, G. & Molenberghs, G. 2000. *Linear Mixed Models for Longitudinal Data*. Springer-Verlag, New York.
- Wingfield, J.C. 1985. Short-term changes in plasma levels of hormones during establishment and defense of a breeding territory in male song sparrows, *Melospiza melodia*. *Horm. Behav.* **19**: 174–187.
- Wingfield, J.C., Ball, G.F., Dufty, A.M. Jr, Hegner, R.E. & Ramenofsky, M. 1987. Testosterone and aggression in birds. *Am. Sci.* **75**: 602–608.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M. Jr & Ball, G.F. 1990. The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems, and breeding systems. *Am. Nat.* **136**: 829–846.
- Wingfield, J.C., Lynn, S.E. & Soma, K.K. 2001. Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *Brain Behav. Evol.* **57**: 239–251.
- Wolf, W.L., Casto, J.M., Nolan, V. Jr & Ketterson, E.D. 2004. Female ornamentation and male mate choice in dark-eyed juncos. *Anim. Behav.* **67**: 93–102.
- Yeh, P.J. 2004. Rapid evolution of a plumage pattern following population establishment in a novel habitat. *Evolution* **58**: 166–174.
- Zera, A.J. & Harshman, L.G. 2001. The physiology of life-history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**: 95–126.

Received 25 September 2007; revised 8 October 2007; accepted 14 October 2007