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A condition dependent link between testosterone and disease resistance in the house finch

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Testosterone has recently been proposed as a link between male quality and health and the expression of sexual traits. We investigated the relationship between testosterone and measures of the individual condition and health of males in a natural population of house finches (*Carpodacus mexicanus*). We also conducted a captive experiment in order to test for the effects of testosterone on resistance to coccidia, which is a common parasite of house finches. Free-living males in better condition had higher testosterone levels and lower corticosterone levels than free-living males in poor condition. In our captive experiment, increased testosterone accelerated the rate of coccidial infection as compared with sham-implanted or gonadectomized males. Although the differences were not significant, free-living males infected with coccidia had lower levels of testosterone and higher levels of corticosterone than males that were not infected. Thus, experimentally elevating testosterone levels in captive males resulted in a higher percentage of infected males, while free-living males with coccidial infection had low testosterone levels. This apparent discrepancy between captive and free-living males in the association of testosterone and disease may be explained by the condition dependence of testosterone. These results suggest that the testosterone-dependent sexual traits reliably indicate male overall condition and health and, thus, females could benefit from assessing potential mates based on these traits.

Keywords: testosterone; corticosterone; sexual selection; condition dependent; immunocompetence; disease susceptibility

1. INTRODUCTION

Some models of sexual selection predict that female choice should be based on secondary sexual traits that indicate male quality reliably (reviewed in Andersson 1994). Secondary sexual traits that reflect male condition should be particularly useful to females that receive direct benefits from a mate (Andersson 1994). However, it is often challenging to measure correctly the condition dependence of sexual traits such as complex mating displays and behaviours. One solution to this problem is to measure the condition dependence of the physiological processes that are involved in trait production. Investigating the physiological processes of trait production can provide insight into how sexual traits and individual condition can become linked, which is central to understanding the evolution of traits that indicate male quality reliably.

The production of testosterone may serve as such a proximate physiological link if it is directly associated with both male quality and the production of secondary sexual traits. Although abundant evidence exists that the production of sexual traits and behaviours is stimulated by a rise in circulating testosterone levels (e.g. singing behaviour in the house finch *Carpodacus mexicanus* (Stoehr & Hill 2000), fleshy ornaments in the moorhen *Gallinula chloropus* (Eens *et al.* 2000) and nuptial plumage in the superb fairy wren *Malurus cyaneus* (Peters *et al.* 2000)), the link between testosterone production and male condition is not well understood. Individual condition probably plays an important role in the regulation of testosterone production and testosterone-dependent traits (e.g. Wingfield 1987). Examples of a direct association between testosterone and individual condition are rare in free-living animals and the importance of testosterone as a link between male condition and sexual trait elaboration is still unknown.

However, there is evidence that testosterone has a direct effect on a specific aspect of individual condition, namely male health. Research in some vertebrates has shown that testosterone production inhibits the immune response and increases an individual's susceptibility to disease (see Marsh 1992 for a review). Folstad & Karter (1992) suggested that the costs males incur through testosterone-mediated immunosuppression force males to regulate their production of testosterone according to their genetic resistance to parasites. Specifically, they suggested that males will self-regulate their testosterone levels in response to parasitic infection. Thus, males infected with parasites should lower their testosterone levels in order to avoid its immunosuppressive effects. In order to simplify their model, Folstad & Karter (1992) minimized the role of overall condition in disease resistance. However, it is well known that overall condition affects an individual's ability to resist disease and, thus, assessing measures of individual condition may provide important insight into the relationship between testosterone and disease resistance.

Testosterone-dependent traits' ability to reflect male quality has been difficult to assess due to the complex nature of the endocrine system (Hews & Moore 1997). Interactions with other hormones such as corticosterone often confound interpretation of these relationships (Evans *et al.* 2000). Elevated corticosterone levels typically indicate physiological stress (Siegel 1980) and

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can reflect an individual's health (Chernin & Morinan 1985), as well as nutritional condition (Kitaysky *et al.* 1999).

In this study, we examined interactions between testosterone, individual condition and disease in both free-living and captive male house finches (*C. mexicanus*). If the ability to elevate testosterone is condition dependent in free-living males, then only males in good condition should be able to elevate their testosterone levels. Thus, we predicted that their testosterone levels would be positively associated with our measures of individual condition (haematocrit and body mass controlled for size). We also investigated the association between testosterone levels and infection by coccidia, which is a common parasite of wild house finches. We predicted that, if testosterone suppresses the immune response, free-living males infected by coccidia would have low testosterone levels. This prediction is based on the assumption that males have the ability to self-regulate their testosterone levels to match their current condition and disease status. We experimentally tested the effects of testosterone on disease in captive males. We predicted that, if testosterone suppresses the immune response, testosterone treatment would increase susceptibility to disease. Finally, we assessed corticosterone levels in order to investigate its role as a confounding variable.

2. METHODS

(a) *Natural population*

The house finch is a sexually dimorphic North American cardueline finch that breeds from late February through to mid-August (Hill 1993). Testosterone influences singing behaviour (Stoehr & Hill 2000) and dominance (R. A. Duckworth, unpublished data) in male house finches. We captured 153 male house finches in traps at feeders during January and February 1999 and 2000 (during the peak of pairing) in Lee County, Alabama. Each bird was banded with a United States Fish and Wildlife Service metal band and a unique combination of three coloured leg bands for individual recognition. We measured tail length, wing cord and tarsus length to the nearest 0.01 mm using calipers, and body mass to the nearest 0.1 g on an electronic balance.

We obtained a blood sample from each male by puncturing the brachial vein and collecting *ca.* 200 µl of blood in heparinized haematocrit capillary tubes in order to assess their hormone levels. We obtained blood samples within 10 min of our approach to the trap in order to minimize the effect of stress on the birds' testosterone levels, and most samples were obtained within 2–5 min of approaching the trap. Birds were left in the trap up to 1 h before sampling. However, this amount of time in the trap did not appear to increase their stress levels above the levels of males bled immediately upon entering the trap (see § 3a). The samples were centrifuged at 2700 rpm for 5 min and the haematocrit, which is the percentage of blood volume that is packed red blood cells, was measured for a subset of males. An increase in haematocrit results in an increase in the oxygen-carrying capacity of blood (Birchard 1997) and is often used as a measure of individual condition (Moreno *et al.* 1998; Ots & Hõrak 1998; Potti *et al.* 1999). All plasma was stored at −20 °C until it was analysed for testosterone and corticosterone levels by radioimmunoassay.

We obtained faecal samples from a subset of males in order to screen for the presence of coccidia, which is an intestinal parasite. We stored the samples in a solution of potassium dichromate and later scored them for the presence of coccidial oocysts using the faecal float method outlined in Brawner *et al.* (2000).

(b) *Captive population*

We tested the effects of testosterone manipulation on captive male house finches that were inoculated with coccidia (*Isospora* spp.). We caught hatch-year male house finches in basket traps at feeders in Lee County, Alabama, during August and September 1999 in order to form the captive flocks. Only hatch-year males were used in order to lessen the chances of prior exposure to coccidial infection. The birds were screened for coccidial infection, *Mycoplasma gallisepticum* and pox lesions and only uninfected birds were retained for the experiment. We retained 36 males for use in our captive experiment.

The birds were housed in groups of seven to eight birds per cage in large outdoor cages and fed a diet of vitamin-enhanced water, sunflower seed and millet *ad libitum*. We added sulphadimethoxine ($1.25\,g\,l^{-1}$) to the drinking water in order to suppress premature coccidial outbreak (Brawner *et al.* 2000). Males were held in these flocks for at least 3 months prior to the beginning of the study in order to ensure that they all remained free of disease.

The birds were randomly assigned to a low testosterone, gonadectomized group (G males), a high testosterone group (T males) or a sham-treated group (sham males). We began our captive experiment with 13 T males, 10 G males and 13 sham males. Three T males died and three lost their implants before the experiment ended. We thus obtained total data for seven T males. One male died in each of the G male and sham male groups, thereby resulting in total data for 9 and 12 birds in these groups, respectively. The G males were anaesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL, USA) and their gonads removed via surgical aspiration in mid-January. These males were closely monitored for 2 weeks following surgery in order to ensure that they recovered fully from surgery and, at the end of this period, all individuals were healthy and vigorous. Both the sham and T males received similar surgery in which their gonads were viewed but left intact. We implanted males in early February 2000, coinciding with the onset of breeding in Alabama populations of house finches (Hill 1993). Each T male received one subcutaneous Silastic tubing implant (Fisher Scientific, Pittsburg, PA, USA) of 8 mm length (1.95 mm outer diameter and 1.47 mm inner diameter), 6 mm of which was filled with crystalline testosterone (Sigma, St Louis, MO, USA). Empty capsules of the same dimensions were implanted into the sham and G males. We returned the birds to their cages after surgery and maintained them in mixed flocks with two to three birds from each treatment group per cage. Mixed flocks were used in order to control for the possibility that attributes of the different cages would bias our results.

Approximately 1 week after the formation of the treatment groups all males were orally inoculated with *ca.* 2000 coccidial oocysts (obtained from naturally infected house finches) suspended in a saline solution. Each male received an oral inoculation of 0.2 ml of the oocyst solution using a syringe with a feeding needle attached. We obtained a faecal sample 1 week following inoculation, which we later screened for the presence of coccidial oocysts (see Brawner *et al.* (2000) for details of these methods).

We bled the G and T males 6 weeks after the implant surgeries in order to confirm our hormonal manipulations.

Approximately 200 µl of blood were collected from each male as outlined in § 2a. Plasma was stored at −20 °C for later hormone analysis. The testosterone manipulations should have produced consistently high testosterone levels in the T males (Duckworth 2000; Stoehr & Hill 2000) and consistently low testosterone levels in the G males (Duckworth 2000). Thus, we sampled these individuals in order to confirm that these manipulations had produced the desired testosterone levels. We did not bleed sham males because a single sampling of circulating testosterone levels in these males was unlikely to reflect the range of testosterone levels exhibited throughout the study. However, in a pilot study, intact males that were treated similarly to our sham males had mean (±s.e.) testosterone levels of 0.32 ± 0.64 ($n = 23$) (see Duckworth 2000). Therefore, we assumed that the sham males in this experiment had relatively low levels of testosterone as compared to the testosterone-implanted males.

(c) *Radioimmunoassay technique*

For a detailed description of the radioimmunoassay technique see Mendonça *et al.* (1996). We used testosterone and corticosterone antibodies from Endocrine Sciences (Calabasas Hills, CA, USA). The assay statistics for free-living males are as follows: the intra-assay variations for testosterone and corticosterone were 3.3 and 5.5%, respectively and the interassay variations were 15.1 and 12.1%, respectively. The average recovery was 84% for testosterone and 82% for corticosterone. The assay sensitivity was 10 pg ml^{-1} for testosterone and 15 pg ml^{-1} for corticosterone. All samples for the captive males were run in one assay that was sensitive to 13 pg ml^{-1}. The intra-assay variation was 3.0% and the average recovery was 85%.

(d) *Statistical analyses*

Individual condition was estimated as the residual of a regression of body mass on the first linear principal component of the tarsus, wing and tail lengths (Rising & Somers 1989). The testosterone and corticosterone values were log transformed and the haematocrit (percentage) values were arcsine transformed before statistical analyses. The corticosterone levels were influenced by both the amount of handling time and the time of day the sample was obtained. We therefore included these variables as covariates in the analyses involving corticosterone. We used partial regression analyses for testing the relationships between hormones and measures of condition. All measures were standardized to a mean of zero and a standard deviation of unity before the analyses. We compared the hormone levels of birds free of disease with those of birds infected with coccidia. Only birds testing negative for coccidian, *M. gallisepticum* and pox (three common diseases in our study population) were included in the disease-free group. We analysed the relationship between disease and hormone levels using a non-parametric Wilcoxon rank sum test and used χ^2 contingency table analyses for determining the effects of testosterone manipulation on coccidial infection in captive males.

3. RESULTS

(a) *Natural population*

The testosterone levels of the birds did not vary with the time of day at which capture occurred (Pearson $r = -0.02$, $p = 0.81$ and $n = 153$) or the amount of handling time ($r = 0.05$ and $p = 0.59$), whereas the corticosterone levels of the birds were related to both the time of capture ($r = -0.27$, $p = 0.03$ and $n = 153$) and the amount of handling time before bleeding ($r = 0.48$ and $p = 0.005$). Thus, we included handling time and time of day as covariates in the analyses involving corticosterone. The birds were in the traps for up to 1 h before blood sampling. However, this did not affect their corticosterone levels ($t_{36} = 0.20$ and $p = 0.84$). The mean (±s.e.) level of corticosterone in males bled immediately upon entering the trap was 5.89 ± 0.65 ng ml^{-1} ($n = 6$), while males that were bled after spending 30–60 min in the trap had mean (±s.e.) corticosterone levels of 6.68 ± 1.39 ng ml^{-1} ($n = 32$).

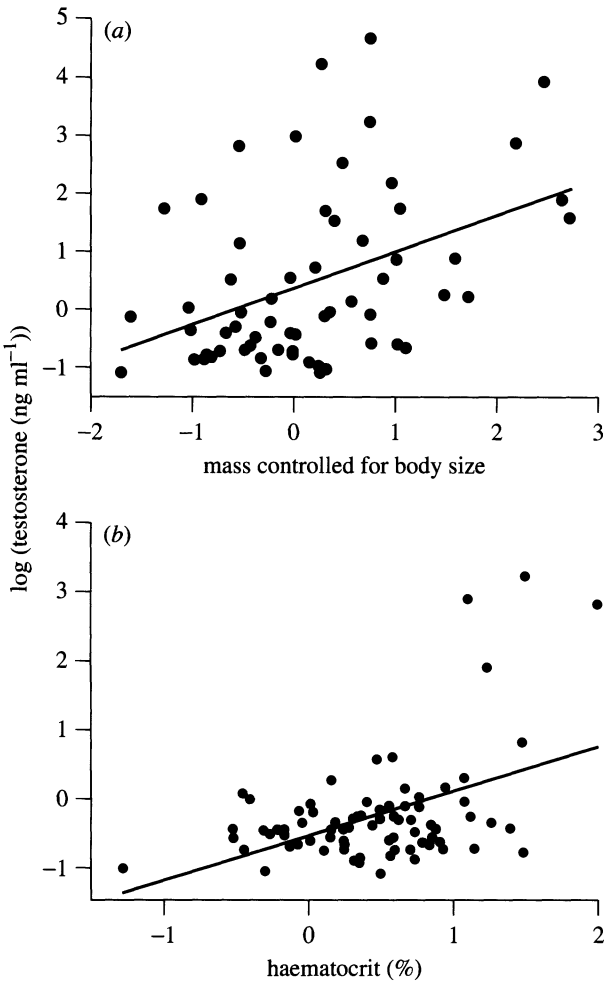

Figure 1. Regression plots illustrating the relationship between log-transformed testosterone levels and individual condition as measured by (*a*) body condition (the residuals of a regression of body mass on the first linear principal component of tarsus, wing and tail lengths) and (*b*) haematocrit percentage (the values are arcsine transformed).

The birds' testosterone levels were positively correlated with the residuals of body mass and body size (standardized regression coefficient $b_{ST} = 0.42$, $p = 0.0007$ and $n = 153$) (figure 1*a*) and haematocrit ($b_{ST} = 0.46$, $p < 0.0001$ and $n = 89$) (figure 1*b*), whereas their corticosterone levels were negatively associated with the residuals of body mass and body size ($b_{ST} = -0.24$, $p = 0.01$ and $n = 153$) (figure 2*a*) and haematocrit ($b_{ST} = -0.21$, $p = 0.005$ and $n = 89$) (figure 2*b*). Corticosterone and testosterone were significantly negatively correlated ($r = -0.21$ and $p = 0.03$). However, this correlation was due to the common effects of individual condition. Using a

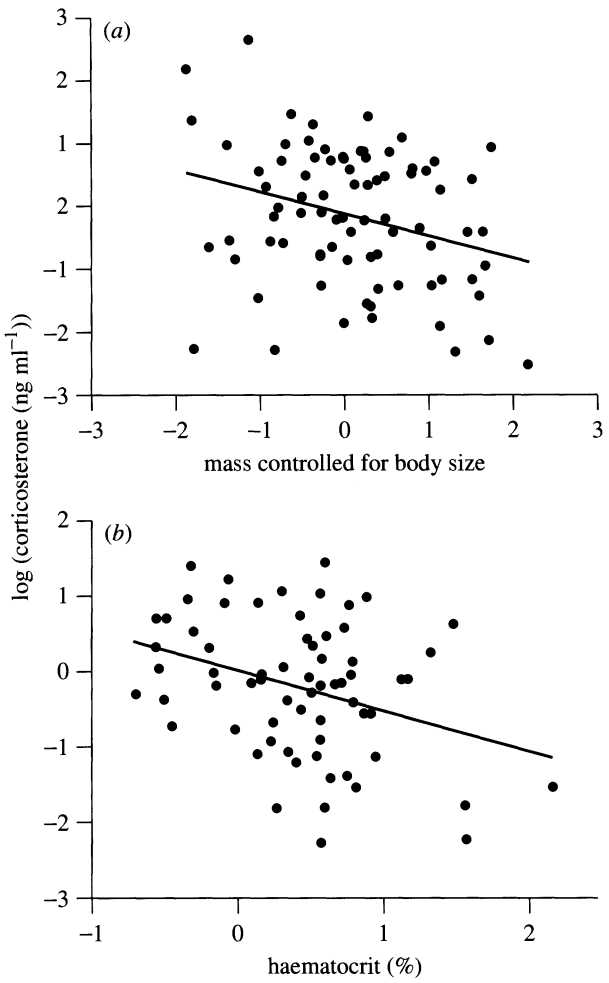

Figure 2. Regression plots illustrating the relationship between corticosterone levels and individual condition as measured by (*a*) body condition (the residuals of a regression of body mass on the first linear principal component of tarsus, wing and tail lengths) and (*b*) haematocrit percentage (the values are arcsine transformed). The corticosterone values are log transformed and handling time and time of day are included as covariates.

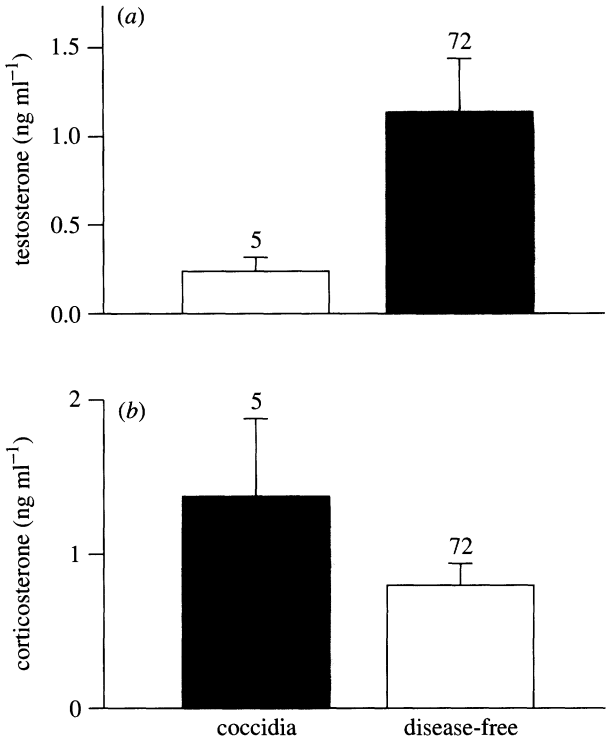

Figure 3. (*a*) Testosterone levels and (*b*) corticosterone levels in males infected with coccidia and males free of disease. The numbers above the error bars refer to sample sizes.

multiple regression for controlling for the effect of haematocrit made the correlation of corticosterone with testosterone non-significant ($b_{ST} = -0.13$, $p = 0.17$ and $n = 89$), while the association of testosterone with haematocrit remained significant ($b_{ST} = 0.48$ and $p = 0.001$). Five of the 77 birds that were screened for coccidia showed signs of infection. The mean (±s.e.) levels of testosterone were lower in birds testing positive for coccidia (0.24 ± 0.06 ng ml^{-1} and $n = 5$) than in birds free of disease (1.13 ± 0.24 ng ml^{-1} and $n = 72$) (figure 3*a*). However, due to the high variance in the testosterone levels of disease-free birds this relationship was not statistically significant ($z = -1.62$ and $p = 0.10$). Birds testing positive for coccidia also had higher mean (±s.e.) corticosterone levels (11.76 ± 3.05 ng ml^{-1} and $n = 5$) than birds free of the disease (8.42 ± 0.95 ng ml^{-1} and $n = 72$) (figure 3*b*); however, this difference was also not statistically significant ($z = 1.25$ and $p = 0.20$).

(b) *Captive population*

The T males averaged (±s.e.) 12.23 ± 2.07 ng ml^{-1} of testosterone and the G males averaged (±s.e.) 0.18 ± 0.35 ng ml^{-1} of testosterone. Thus, the average testosterone levels exhibited by the T males were high but within the range of testosterone levels found in free-living house finches. Free-living males have a mean (±s.e.) testosterone level of 1.17 ± 0.24 ng ml^{-1}. However, testosterone levels as high as 15 ng ml^{-1} have been recorded. We found no oocysts in a random pre-experiment screening of the faecal samples from the captive males, thereby confirming that the sulphadimethoxine treatment had effectively suppressed coccidial infection. There was no effect of cage on the prevalence of coccidial infection ($p > 0.05$) or on the testosterone levels ($p > 0.05$). We thus treated males as individual data points in our statistical analyses. The T males were more susceptible to coccidial infection (100% infected) than either the sham (41% infected) or G males (22% infected) ($\chi^2 = 12.41$ and $p = 0.001$) as measured by the presence of coccidial infection 1 week after inoculation (see table 1). A higher percentage of sham males than G males were infected; however, this trend was not significant ($\chi^2 = 2.85$ and $p = 0.09$) (table 1). There was no difference in oocyst output among the infected males of the treatment groups ($F_{2,27} = 0.23$ and $p = 0.75$) (table 1).

4. DISCUSSION

In this study, we have documented that testosterone was positively correlated with two measures of individual condition in a natural population. We also found that males infected with coccidia exhibited low levels of testosterone as compared with males free of disease, although this difference was not statistically significant. Thus, free-living males with high levels of testosterone were in better condition and tended to have a lower probability of

Table 1. The effects of testosterone manipulation on susceptibility to coccidial infection in captive male house finches.

(The T males received testosterone-filled implants, the G males had their gonads removed and received empty implants and the sham males received empty implants. There was a significantly higher percentage of T males infected than sham or G males ($\chi^2 = 12.41$ and $p = 0.001$). However, the difference between sham and G males was not statistically significant ($\chi^2 = 2.85$ and $p = 0.09$). There was no difference in the severity of infection among infected males ($F = 0.23$ and $p = 0.75$).)

treatment group	*n*	percentage infected	mean oocysts in infected males (*n*)
T males	7	100	2.78 (7)
Sham males	12	41	2.84 (5)
G males	9	22	2.34 (2)

coccidial infection as compared with males with lower levels of testosterone. We found the opposite relationships for corticosterone, condition and disease. Males with high levels of corticosterone were in poorer condition and tended to have a higher probability of coccidial infection than males with low levels of corticosterone. Finally, in the captive study, artificial elevation of testosterone levels increased the number of individuals exhibiting signs of coccidial infection, while the low testosterone groups had few infected individuals.

To the authors' knowledge, this is the first study to demonstrate a relationship between testosterone and individual condition in a natural population of birds. The positive association suggests that individual condition directly affects testosterone production. Experimental evidence from captive studies supports this interpretation. For example, the direct manipulation of individual condition through food deprivation produced a rapid decrease in the testosterone levels of captive white-crowned sparrows (*Zonotrichia leucophrys*) (Wingfield 1987). In addition, the expression of testosterone-dependent traits in captive zebra finches (*Taeniopygia guttata*) was positively related to individual condition in experimentally stressed males (Birkhead *et al.* 1998). Although the positive association we found suggests that individual condition directly affects circulating testosterone levels, it is possible that testosterone and individual condition are linked indirectly through a third factor, such as corticosterone. However, statistically controlling for the effects of corticosterone did not change the positive association between testosterone and condition. This supports a direct relationship between testosterone and individual condition, providing further evidence that testosterone is condition dependent in the house finch. In order to determine unequivocally whether individual condition directly affects testosterone production, it will be necessary to conduct experimental studies in which individual condition is manipulated and changes in testosterone levels are measured.

The results of our captive experiment, in which we controlled for differences in individual condition (males were randomly placed in treatment groups and were maintained in a homogeneous environment), suggest that treatment with testosterone has direct negative effects on parasite resistance. These results are consistent with other studies in which experimental elevation of testosterone resulted in higher levels of parasitic infection (Saino *et al.* 1995; Zuk *et al.* 1995). We found the opposite relationship between testosterone and coccidial infection in free-living house finches, where low testosterone levels were associated with the presence of coccidial infection. Although the testosterone levels in diseased and healthy free-living males were not significantly different, the lower testosterone levels in diseased males is consistent with our *a priori* prediction that testosterone would be lower in diseased males due to individual self-regulation of testosterone levels. Thus, the relationship between testosterone and disease in free-living versus captive house finches warrants further investigation. It is likely that the testosterone levels in captive males are elevated above each individual's optimal level (this notion is supported by the evidence that very few free-living individuals maintain testosterone levels above 10 ng ml^{-1}) (see Duckworth 2000 for details), and, thus, the costs of testosterone become apparent in these individuals. However, because free-living males can self-regulate their testosterone levels, both the individual condition and disease status of a male will determine the level and extent that testosterone elevation is possible. Males in good condition are able to elevate their testosterone levels without suffering the consequence of parasitic infection, while males in poor condition are unable to elevate their testosterone levels due to the added cost of immunosuppression. Thus, we see opposite relationships between testosterone and disease when testosterone is experimentally elevated versus situations in which males can self-regulate their testosterone levels. It would be interesting to see whether individual condition mediates the relationship between testosterone production and disease as has been suggested elsewhere (Peters 2000). Unfortunately, we only obtained data on individual condition for a few of the males on which we also had coccidia data and, thus, we were unable to test for differences in individual condition among infected and non-infected males.

These results shed light on previous studies that have investigated the relationship between testosterone levels and parasite resistance yet produced inconclusive results. Researchers typically find no association between parasite loads and testosterone levels in natural populations (Weatherhead *et al.* 1993; Saino & Møller 1994). In contrast to these correlational field studies, in studies in which testosterone levels were manipulated, testosterone treatment increases parasite loads (Saino *et al.* 1995; Eens *et al.* 2000; Poiani *et al.* 2000). Without taking differences in individual condition into account it may be difficult to discern a relationship between dynamic traits such as hormone levels and parasite loads in a natural population. In contrast, we would expect the costs of testosterone elevation to be more evident in experimental manipulations because the testosterone levels are elevated irrespective of differences in individual condition.

Females could benefit in many ways by assessing male quality based on testosterone-dependent traits. In the house finch, testosterone-dependent traits include both their song rate (Stoehr & Hill 2000) and dominance

status (R. A. Duckworth, unpublished data). By paying attention to these traits, females could gain information about multiple factors regarding a prospective mate including overall condition and parasitic infection. Furthermore, testosterone levels can change in a very short time when males are experiencing environmental stress (Wingfield 1987). Thus, testosterone-dependent traits should be particularly useful in reflecting a male's immediate condition. These factors may be particularly important for females of a monogamous species with obligatory biparental care such as the house finch, in which male condition and health may have a strong direct effect on female fecundity and survival.

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