

## GENIC CAPTURE AND THE GENETIC BASIS OF SEXUALLY SELECTED TRAITS IN THE ZEBRA FINCH

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**Abstract.**—The lek paradox, in which female choice erodes genetic variation in male sexually selected traits, is a fundamental issue in sexual selection. If females gain only genetic benefits from preferentially having their ova fertilized by males with particular traits, what maintains variation in these traits? Under strong directional selection mediated through mate choice, the alleles for beneficial male traits are expected to go to fixation and exhibit little variation. A theoretical solution to the lek paradox is the genic capture hypothesis which states that: costly male traits subject to female choice are condition dependent, that male condition is dependent on genes at many loci and exhibits additive genetic variance, and that positive genetic correlations exist between sexually selected traits and condition. Using a captive population of the zebra finch *Taeniopygia guttata*, we tested two key predictions from this model: (1) that genetic variance exists in beak color which is a sexually selected trait, but also in condition and immune function, and (2) that positive genetic correlations exist between condition and beak color, and between beak color, condition, and immune function. Genetic parameters were estimated from a large breeding experiment involving 81 sires, 972 offspring, a pedigree of 1526 individuals, using the animal model. We employed the following index of body condition: residuals from a log-log plot of body mass on tarsus length following a standardized and extended period of exercise, in which residual mass is known to reflect fat and protein reserves. Our results were broadly consistent with the genic capture hypothesis because we found (1) additive genetic variation in beak color and immune function and condition, and (2) positive genetic correlations between condition and beak color, and between condition, beak color, and several assays of immune responsiveness. However, both of these results need qualification. In the first case we identified an important general problem in estimating the coefficient of additive genetic variance ( $CV_A$ ) in body condition. In the second case, although most of the genetic correlations were positive as predicted, only some were statistically significant, possibly due to our relatively small sample sizes, because genetic correlations typically have large standard errors and therefore require very large samples to be statistically significant. The statistically significant, positive genetic correlations included those between beak color and immune function (response to tetanus), and between immune function (response to tetanus) and condition, both of which indicate that females gain good genes from mating with males in good condition and/or with a redder beak color. We discuss the implications of our results for devising more rigorous but pragmatic tests of the genic capture hypothesis.

**Key words.**—Animal model, heritability, lek paradox, sexual selection, zebra finch.

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The concept of female choice within Darwin's theory of sexual selection has always been controversial when females appear to gain only indirect (i.e., genetic) benefits from their choice of partner (Andersson 1994). The central problem is the maintenance of additive genetic variation in traits subject to strong, directional sexual selection: if most females prefer males with particular traits because they confer genetic benefits on a female's offspring, why have these traits not gone to fixation? (Fisher 1930; Borgia 1979; Kirkpatrick and Ryan 1991). This evolutionary puzzle has been referred to as the lek paradox and is epitomized by species with a lek mating system in which females gain no obvious direct benefits from their choice of partner, and by default must obtain indirect benefits. A similar situation exists in socially monogamous birds in which females engage in extrapair copulations and apparently gain no direct benefits from their choice of extrapair partner (Birkhead 1998; Griffith et al. 2002). In both lekking systems and in socially monogamous systems with high levels of extrapair copulations, sexual selection through

female choice for male traits is likely to be intense. The paradox is that despite intense directional sexual selection the variation in male traits is not eroded.

Several solutions to the lek paradox have been proposed. For example, it has been suggested that fluctuating selection, such as occurs through host-parasite coevolution, results in optimal phenotypes varying in either space or time (Hamilton and Zuk 1982). Another solution is the genic capture hypothesis (Houle 1992; Iwasa and Pomiankowski 1994; Rowe and Houle 1996; Tomkins et al. 2004) which is based on the concept of mutation-selection balance, that is, condition depends upon so many alleles that mutations arise as rapidly as selection removes them. The genic capture hypothesis rests on two main assumptions: (1) secondary sexual traits are condition dependent, and (2) there is additive genetic variation in secondary sexual traits and in traits such as body condition and other traits that affect viability, such as immune capacity (Iwasa and Pomiankowski 1994; Rowe and Houle 1996; Tomkins et al. 2004). It is well known to animal breeders that body condition and fat scores are heritable (e.g., Smith et al. 1962) and several studies have provided evidence that immunity, condition, and male sexual ornaments are heritable in birds and other organisms (Bakker 1993; Gustafsson et al. 1994; Pomiankowski and Møller 1995; Brinkhof et al. 1999; Qvarnström 1999; David et al. 2000; Coltman et al.

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2001; Kotiaho et al. 2001; Johnsen et al. 2003; Lorch et al. 2003; Soler et al. 2003; Garant et al. 2004).

The genic capture hypothesis has been difficult to test, partly because of the elusive nature of the concept of condition (Brown 1996; Kotiaho 1999; Blakenhorn and Hosken 2003). Condition has been defined as "the pool of resources available for utilisation" and equivalent to residual reproductive value or "the amount of resources available for allocation to fitness-enhancing traits" (Kotiaho 1999). Although the pool of resources available for utilization in an individual is difficult to measure directly, various surrogates or indirect estimates of condition (Brown 1996; Jakob et al. 1996; Kotiaho 1999; Blakenhorn and Hosken 2003) indicate a clear link with the development of sexually selected traits (Andersson 1994; David et al. 2000; Kotiaho et al. 2001).

The predictions of the genic capture hypothesis are as follows (Tomkins et al. 2004): (1) The expression of sexually selected traits is dependent on condition; (2) freed from the costs of producing secondary sexual traits individuals should exhibit improved naturally selected fitness components; (3) condition should have a high coefficient of additive genetic variance; (4) sexually selected traits should exhibit positive genetic correlations with condition; (5) condition and condition dependent traits should be affected by many loci; (6) by increasing the strength of selection on condition, sexual selection should enable populations to adapt more rapidly to environmental changes or to purge genetic load, and (7) traits that are more costly capture more condition-dependent variation.

So far, only some of these predictions have been tested and in only a few studies (David et al. 2000; Kotiaho et al. 2001; Hunt et al. 2004; Rolff et al. 2005). The aim of the present study, using the zebra finch *Taeniopygia guttata* as a model species, was to test predictions (3) and (4). Specifically, with regard to prediction (3), that genetic variance exists in beak color, but also condition and immune function, and with regard to prediction (4) that positive genetic correlations exists between condition and beak color, and between beak color, condition, and immune function.

#### METHODS

The zebra finch is a small (12–17 g), sexually dimorphic passerine bird in which sexual selection has been studied extensively. It is a socially monogamous, colonial passerine bird in which extrapair behavior is frequent and extrapair fertilizations occur both in the wild and in captivity (Birkhead et al. 1988a,b, 1990). Because female choice of both long-term and extrapair partners appears to be based on beak color (Zann 1996; Blount et al. 2003; but see Forstmeier and Birkhead 2004) and because it is brighter in males than females (Zann 1996), beak color is assumed to be a sexually selected trait in the zebra finch. We used domesticated, wild-type zebra finches, which were part of an outbred study population based at the University of Sheffield since 1985. The entire pedigree comprises over 6000 birds originating from 181 male and 155 female ancestors, and the mean  $\pm$  S.D. inbreeding coefficient of the birds used in the present study was  $0.030 \pm 0.0139$  (calculated using Pedsys; L. Keller, pers. comm.). We maintained birds on a 14:10 h light:dark regime

at  $\sim 20^\circ\text{C}$  on a standard ad libitum zebra finch seed diet and water, supplemented with hard-boiled egg, soaked seed during breeding. Pairs of zebra finches were established in batches of about 30 over a period of 35 months (from September 1999 to July 2002), producing 15 cohorts of young birds, each comprising 100–120 individuals. Breeding pairs were housed individually in cages ( $50 \times 45 \times 46$  cm high), and provided with a nest box on the outside of the cage. Prior to breeding females were isolated from males for at least four weeks to ensure that they carried no sperm from previous males (see Birkhead et al. 1988a). Nest boxes were checked every day; eggs were numbered on the day of laying and the fate of each egg recorded. All eggs were cross-fostered to reduce environmental effects, but because we split broods between several foster parents and were sometimes forced to move chicks between several foster parents, we did not include a foster-parent effect in the analysis. On hatching, chicks were marked for individual identification and ringed at 8–10 days of age. Chicks were removed from their foster parents 37 days after hatching; sex recorded (from plumage), body mass recorded (between 1400–1600 h local time). Male and female offspring were kept in separate cages for two weeks before being placed with other birds of the same sex in their cohort until a total of 120 was reached. The study focused on male offspring.

There has been some discussion regarding the validity of conducting quantitative genetic analyses on captive populations and assuming that estimates will be similar in wild individuals (Weigensberg and Roff 1996). The main concern is that captive populations have lost genetic variability. This is not the case in the present study. We have compared levels of genetic variation in our captive population with those in a wild population from Broken Hill, New South Wales, Australia. Twenty-four birds from both populations have been typed at seven microsatellite loci and show remarkably similar levels of variation: Sheffield, mean number of alleles per locus = 4.14, mean heterozygosity = 0.57; Broken Hill, mean number of alleles per locus = 4.86, mean heterozygosity = 0.61; these differences are nonsignificant (J. Slate, M. C. Hale, and T. R. Birkhead, unpubl. ms.). In another study of mitochondrial DNA variation, we sequenced the entire mtDNA genome in six birds from our captive population and found five unique haplotypes (Mossman et al. 2006). Therefore, there is no suggestion that our population or its source populations have been through a bottleneck.

*Estimating genetic parameters.*—We used a combined full-sibling and half-sibling breeding design. Sires were unrelated as far back as grandparents and dams were unrelated to their male partner (back as far as grandparents) and all the females paired to a particular breeding male were unrelated to each other (as far back as grandparents). Each female was paired twice, once each with a different male to increase our ability to detect maternal effects. The study focussed on male offspring and traits were measured when birds were aged 168–203 days.

Heritabilities and other genetic parameters were estimated using the animal model (Kruuk 2004; Birkhead et al. 2005) from a combined full-sibling and half-sibling animal breeding design involving 81 sires producing two sons from each of six dams (972 male offspring, 1526 individuals in total) and

analyzed using the multiple trait derivative-free restricted maximum-likelihood program (MTDFREML) (Boldman et al. 1995; see also Birkhead et al. 2005).

The model used to estimate (co)variance components was a multiple trait linear mixed animal model. In matrix notation

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e} \quad (1)$$

where  $\mathbf{X}$  is a known indicator matrix accounting for the fixed effects (cohort, clutch size and dam age, when declared significant)  $\boldsymbol{\beta}$  is the unknown vector of fixed effects,  $\mathbf{Z}$  is a known indicator matrix associating animal effects to the vector of observations  $\mathbf{Y}$ ,  $\mathbf{u}$  is the vector of unknown random animal effects, and  $\mathbf{e}$  is the vector of residual environmental random effects. Subsets of two traits at a time were analyzed. We obtained univariate estimates for genetic and environmental variances using the multiple trait derivative-free REML algorithm implemented in MTDFREML software and using them as starting values in the bivariate analysis (Boldman et al. 1995). Convergence criterion was attained when the variance of the simplex values was  $\leq 10^{12}$ . It was assumed that global maximum was obtained when two restarts, using previous converged values as starting values, produced convergence with no changes in the first three decimal places of the  $F$ -value (Boldman et al. 1995). Linear models were used to identify important sources of fixed effect variation for each trait. Terms fitted as fixed effects were dam age (quantitative continuous), clutch size (two to 11), number of fertile eggs (one to 10) and cohort (15 class categories), but only some of these fixed effects were significant for some traits.

We tested whether the heritabilities and genetic correlations were significantly different from zero by: (1) estimating the standard error of each estimate from the  $P$ -value obtained from the likelihood ratio test (LRT). This procedure is simply an extrapolation (chi squared to normal) based on the normal distribution. The results were checked using ASReml software; they yielded practically identical standard errors; (2) significance levels were calculated from the LRT.

*Measurement of traits.*—Male beak color is considered a condition-dependent, sexually selected trait in the zebra finch (Zann 1996). The zebra finch is highly sexually dimorphic in both plumage and beak color: males have a bright red beak (females have an orange beak) (Morris 1954; Zann 1996), and in several studies of captive/domesticated birds females prefer males with redder beaks and higher song rates (summarized in Zann 1996; see also Fortstmeier and Birkhead 2004). Not all studies have found a female preference for beak color however, and one study using birds from the present population found no effect of natural variation in beak color on female preferences (see Fortstmeier and Birkhead 2004). However, in another study also of the present population, in which male diets were supplemented with carotenoids and whose beaks became redder as a result, females showed a strong preference for these males (Blount et al. 2003). Domesticated birds on an ad libitum diet may show less variation in beak color than wild birds, and certainly among wild birds there is good evidence that individuals in poor condition have less red beaks than birds in better condition (Burley et al. 1992; Zann 1994, 1996).

In the present study beak color was measured under standardized conditions on a scale from 0 (orange) to 6 (dark

red) using Munsell color chips as in our previous studies. Color scores correlate closely with objectively measured spectradiametric measures (Birkhead et al. 1998). Bill color was measured within five days of the start (beak before) and five days of the end (beak after) of the exercise period.

*Body condition.*—Condition is an elusive concept. It is also confusing since the term has been used in different ways. Condition has been used to refer to an individual's phenotypic quality or long-term viability (Andersson 1982; Hunt et al. 2004), but it has also been used to refer to body condition or relative fatness, which until recently was assumed to reflect an individual's nongenetic nutritional status (Merilä and Sheldon 2001) and short-term viability. During a period of food shortage for example, an individual with more body reserves may be more likely to survive than one with fewer reserves (Brown 1996). Although body condition is susceptible to environmental variation, in fledgling flycatchers *Ficedula albicollis* and blue tits *Parus [Cyanistes] caeruleus*, it has a pronounced genetic component and predicts their likelihood of survival (Merilä 1996; Sheldon et al. 1997; Merilä et al. 1999, 2001). However, among adult birds of some species individuals with relatively large body reserves may be subordinates, and their greater relative mass may make them more vulnerable to predation (Witter and Cuthill 1993; Gosler and Harper 2000; Cresswell 2003).

Body condition can be estimated in two main ways, either as resource acquisition or the standing pool of resources. Resource acquisition can be measured through growth rate, which encompasses foraging, digestion, and metabolic efficiency (Hunt et al. 2004; Tomkins et al. 2004). The pool of resources available to an individual is typically estimated as mass corrected for body size, such as the ratio of cube root of body mass:tarsus length or residuals from a regression of body mass on tarsus length (Brown 1996; Green 2001). Both measures can provide biologically sensible estimates of the amount of reserves such as fat or protein that can be converted into energy, but they have different statistical attributes. Ratio data and residuals have several statistical limitations (Packard and Boardman 1987; Sokal and Rohlf 1995; Kotiaho 1999; Green 2001; Freckleton 2002) but of the two, residuals are preferred, providing some key assumptions are met (Garcia-Berthou 2001; Green 2001; Tomkins and Simmons 2002; Schult-Hostedde et al. 2005). These assumptions include the following: (1) the functional relationship between mass and the body size index (in the present study, tarsus length) is linear; (2) residual mass (the condition index) is independent of the body size index; (3) the body size index accurately reflects structural size; (4) body size is measured without error; and (5) that residual mass reflects genuine variation in the pool of resources and not simply variance in shape or water content, which can be verified either by demonstrating that the absolute amount of fat or other reserve is positively correlated with residual mass or by manipulating residual mass experimentally through diet quality (e.g., David et al. 2000), food deprivation or starvation (e.g., Kotiaho et al. 2001). Relatively few of the studies that have used residual mass as an index of condition have verified that residual mass is positively and significantly correlated with reserves (see Piersma 1984; Green 2001; Ardia 2005).

In the present study we estimated body condition as re-

siduals from a log-log regression of body mass on tarsus, after experimentally manipulating residual mass through a prolonged (six weeks) and standardized period of exercise (see below), which (as predicted on the basis of our previous work (Birkhead et al. 1998)) resulted in a significant reduction in body mass (mean difference:  $-0.444 \text{ g} \pm 0.032 \text{ SE}$ ; paired  $t$  test,  $t = 13.91$ ,  $df = 971$ ,  $P < 0.0001$ ). We used residuals from a log-log regression to deal with the fact that a unit of reserves may be more valuable to a smaller individual than a larger one (Kotiaho 1999). We also checked and verified the above assumptions regarding the use of residual mass as an index of body condition, thus: (1) the relationship between mass and tarsus is linear; this was achieved using the log.log. relationship; (2) condition is independent of tarsus length: adding (tarsus)<sup>2</sup> to the relationship between condition and tarsus did significantly ( $P < 0.01$ ) improve the fit in a multiple regression, but the tarsus squared term had a negligible effect and explained only an additional 0.63% of the variation. Although statistically significant (a consequence of the large sample size ( $n = 956$ )), effectively the relationship is very close to linear; (3) tarsus length accurately reflects structural size: we dissected a sub-sample of 45 birds and made three skeletal measurements of body size (keel length, keel depth and the linear distance between the proximal end of the humerus and the tip of the keel), and then used principle components analysis to combine these three measures: PC1 explained 86% of the variance in size and was significantly correlated with tarsus length (Spearman rank correlation  $r_s = 0.727$ ,  $P < 0.0001$ ); (4) tarsus length is measured without error. Tarsus length was not measured without error: however, all tarsus measurements were made by the same person, and a subsample of tarsi from 20 birds taken at random and measured blind three times to check for observer consistency showed high repeatability: 0.98 ( $P < 0.001$ ), indicating that measurement error was small; (5) residual mass reflects variation in an individual's pool of resources: using a subsample of 60 birds we found that residual mass was positively and significantly associated with both fat score (Kruskal-Wallis  $\chi^2 = 17.81$ ,  $df = 2$ ,  $P < 0.001$ ) and the dry mass of the pectoral muscles (Spearman rank correlation  $r_s = 0.800$ ,  $P < 0.001$ ), indicating that birds in good condition had both more fat and breast muscle protein.

In a previous study we manipulated zebra finch body condition through diet quality in nestlings, but found that this had little effect on adult body mass or beak color, possibly because birds traded beak color against other fitness traits (Birkhead et al. 1999). We did not consider food deprivation appropriate in the present study for humane reasons, but also because if birds are effectively starving (as they probably were in Lemon's (1991) study for example), any investigation becomes meaningless. As well as being more realistic, our manipulation of residual body mass through an exercise regime eliminated the likelihood of encountering obese individuals. It also reduced the chances of short-term fluctuations in body reserves, and because all birds were subject to the same amount of exercise, removed much of the variation in mass attributable to individual differences in activity levels. In other words, this assay avoided some (but probably not all) of the problems associated with the use of residual body mass as an index of body condition in wild birds. As in other

studies, we do not know whether our index of body condition reflects long-term fitness.

The assay was performed as follows: cohorts were trained to fly 4 km day<sup>-1</sup> for six weeks by a person moving repeatedly from one end of the aviary to the other in four bouts per day, five days per week. After an initial training period of five days in which the distance flown each day increased from 0.36 km to 3.0 km, birds were thereafter encouraged to fly 4 km day<sup>-1</sup>. Combining this exercise with the birds' normal activity ( $\sim 0.87$  km, measured by video taping all birds between exercise bouts for two entire days), the mean distance flown each day during the exercise period was 4.87 km day<sup>-1</sup>. Body mass was measured on the day before the start of the exercise period (see below) and the day it ended. Body mass was measured (to the nearest 0.1 g) using an Ohaus LS200 electronic balance between 1400–1600 h local time (3–4 h before the lights went off in the evening) to minimize the effects of diurnal variation in body mass (Metcalf and Ure 1995). Tarsus length was measured after the birds had been humanely killed at the end of the study (as required by the license under which the study was conducted). The left tarsus was removed and measured to the nearest 0.01 mm with Vernier callipers, and as mentioned earlier was highly repeatable.

It has been suggested that hematocrit (packed cell blood volume) may also serve as an index of phenotypic condition (Saino et al. 1997). In an earlier study we found hematocrit to be highly repeatable within individual zebra finches, to show a reduction in response to long term exercise as predicted (see Green et al. 1991), but to be independent of primary and secondary sexual traits (Birkhead et al. 1998). In the present study, we collected blood samples as part of the humoral immunity measures (see below), and we also measured hematocrit and included it in our analyses for completeness rather than because we anticipated it being a useful index of condition or had any a priori predictions regarding its relationship with other variables.

*Immune capacity.*—An individual's immune capacity can be measured as the strength of immune response to novel antigens and is assumed to reflect the response to a natural infection (Luster et al. 1988; Lochmiller et al. 1993). It has also been suggested that immune capacity is dependent on an individual's nutritional status and may therefore be a condition-dependent trait (Coltman et al. 2001; Soler et al. 2003; Cotter et al. 2004; Rolff et al. 2005). Because different components of the immune system are effective against different types of infection, the most efficient way to assess an individual's response to infection is to expose them to a suite of novel antigens and measure their antibody responses (Luster et al. 1988, 1992; Lochmiller et al. 1993). We used three assays of immune responsiveness: (1) a T-cell mediated response to an intradermal inoculation of phytohaemagglutinin (PHA), sometimes referred to as the wing web index (Goto et al. 1978; Lochmiller et al. 1993) and referred to here as the PHA assay; and two humoral T-dependent immune responses to: (2) human diphtheria vaccine, and (3) human tetanus vaccine, which provided in vivo measures of immunoglobulin, using a mouse antibody against zebra finch measured using an ELISA (see Svensson et al. 1998, McPhee 2000; Råberg et al. 2003). For the PHA assay we measured

TABLE 1. Means and coefficients of variation of zebra finch traits. CVP, phenotypic coefficient of variation; CVA, additive genetic coefficient of variation; CVR, environmental coefficient of variation based on MTDFREML variance component estimates and using an animal model. Only the additive genetic direct effect is included. \*\*  $P < 0.01$ ; \*\*\*  $< 0.001$ .

Trait	Mean	SD	CVP	CVA	CVR
Beak before exercise	4.12	0.74	17.96	9.65	13.77
Beak after exercise	4.17	0.67	16.17	9.06	13.39
Condition <sup>1</sup>	—	—	7.39	5.70	1.93
Hematocrit	51.22	3.58	7.00	4.44	5.41
Immune assays:					
PHA	109.36	18.90	17.28	8.13	15.25
Diphtheria	-1.056	0.304	28.81	11.66	26.34
Tetanus	-0.353	0.245	69.41	24.35	65.09
Adult mass (g)	16.94	1.64	9.69	7.99	5.48
Tarsus (mm)	16.85	0.57	3.38	2.98	1.58

<sup>1</sup> No mean value or SD given because condition is based on residual values. Note also that the values for  $CV_P$  and  $CV_A$  are based on residuals and mean body mass from the untransformed relationship between tarsus and body mass. Using logged values and log body mass to residuals, gave smaller values of  $CV_A$  and  $CV_P$  (see text).

the depth of the patagium on both wings using a spessimeter (accurate to 0.01mm) five times and took the mean of these measurements; we then injected 20  $\mu$ l of PHA diluted in phosphate buffered saline (2.5 mg ml<sup>-1</sup>) into the patagium and 24 h later measured the patagium again five times and then calculated the mean value. All measurements were conducted by the same person whose measurement repeatability was around 0.90. The wing web index was calculated as the difference in thickness of each patagium before and after the PHA injection, minus the difference in the control wing, as in Lochmiller et al. (1993). The PHA assay was conducted 10–30 days before the period of exercise. The combined human diphtheria and tetanus vaccine was administered via intramuscular injection and the secondary responses to both antigens measured separately for each bird. We used the log-

antibody responses in the analyses. The diphtheria and tetanus assays were conducted between seven and 28 days of the end of the exercise period.

## RESULTS

*Genetic variance in beak color.*—The levels of additive genetic variance ( $CV_A$ ) in beak color were moderate (~9%; Table 1) and the heritability estimates of beak color measured before and after the exercise period were similar: 0.312 and 0.332, respectively (Table 2) and within the range of those reported in an earlier study (0.23–0.58; Price and Burley 1993; Price 1996). We also found a strong positive genetic correlation between beak color before and after the period of exercise ( $r = 0.987$ ,  $P < 0.001$ ), indicating that there was

TABLE 2. Genetic variance covariance matrix for traits in the zebra finch associated with beak color (a secondary sexual trait), condition, immunological assays and other traits.

	Beak before	Beak after	Condition	Hematocrit	PHA assay	Diphtheria assay	Tetanus assay	Body mass
Beak before	<b>0.332</b> (0.074) $P < 0.001$	0.987 (0.043) $P < 0.001$	0.387 (0.166) $P < 0.01$	0.115 (0.171) $P = 0.25$	0.178 (0.200) $P = 0.19$	0.252 (0.175) $P = 0.08$	0.635 (0.247) $P = 0.013$	0.094 (0.154) $P = 0.27$
Beak after	0.73 [0.60]	<b>0.312</b> (0.070) $P < 0.001$	0.292 (0.186) $P < 0.06$	-0.073 (0.177) $P = 0.34$	0.239 (0.200) $P = 0.20$	0.311 (0.179) $P = 0.28$	0.750 (0.230) $P < 0.003$	-0.003 (0.023) $P = 0.45$
Condition	0.150 [-0.06]	0.130 [0.01]	<b>0.610</b> (0.050) $P < 0.001$	0.516 (0.151) $P < 0.001$	0.226 (0.251) $P = 0.18$	0.058 (0.235) $P = 0.40$	0.470 (0.214) $P = 0.029$	0.890 (0.10) $P < 0.001$
Hematocrit	0.07 [0.05]	0.00 [-0.04]	0.120 [-0.29]	<b>0.380</b> (0.077) $P < 0.001$	0.082 (0.193) $P = 0.34$	0.364 (0.233) $P = 0.06$	0.646 (0.228) $P < 0.007$	0.424 (0.147) $P < 0.004$
PHA assay	0.04 [0.01]	0.03 [-0.04]	0.06 [-0.04]	0.04 [0.02]	<b>0.221</b> (0.064) $P < 0.001$	-0.538 (0.183) $P < 0.021$	-0.281 (0.211) $P = 0.09$	0.079 (0.172) $P = 0.32$
Diphtheria assay	0.04 [-0.02]	0.03 [-0.06]	-0.03 [-0.09]	0.03 [-0.08]	-0.04 [0.08]	<b>0.161</b> (0.070) $P < 0.001$	0.978 (0.237) $P < 0.001$	-0.075 (0.180) $P = 0.34$
Tetanus assay	0.06 [-0.08]	0.06 [-0.10]	0.04 [-0.14]	0.05 [-0.12]	-0.02 [0.04]	0.54 [0.47]	<b>0.121</b> (0.053) $P < 0.001$	0.398 (0.205) $P = 0.058$
Body mass	0.10 [0.12]	0.09 [0.18]	0.91 [0.94]	0.11 [-0.27]	0.00 [-0.06]	-0.06 [-0.06]	0.06 [-0.011]	<b>0.680</b> (0.207) $P < 0.001$

Diagonal and bold, heritability estimate ( $\pm$ SE). Above diagonal, genetic correlations ( $\pm$ SE). Below diagonal, phenotypic and [environmental] correlations.

TABLE 3. Direct and indirect (genetic maternal) effects for all traits.

Trait	Model 1 $h^2$	Model 2		LRT $P$ value
		$h^2_{\text{direct}}$	$h^2_{\text{maternal}} \pm \text{SE}$	
Condition	0.61	0.38	0.02 $\pm$ (0.014)	0.07
Body mass	0.68	0.54	0.04 $\pm$ (0.048)	0.20
PHA	0.22	0.22	0.01 $\pm$ (0.026)	0.65
Hematocrit	0.38	0.30	0.05 $\pm$ (0.074)	0.25
Beak before	0.33	0.21	0.02 $\pm$ (0.016)	0.11
Beak after	0.31	0.21	0.02 $\pm$ (0.016)	0.10
Diphtheria	0.16	0.08	0.02 $\pm$ (0.021)	0.17
Tetanus	0.12	0.11	0.02 $\pm$ (0.033)	0.73
Tarsus	0.78	0.73	0.10 $\pm$ (0.071)	0.08

LRT, Likelihood ratio test (1 df; as described in Birkhead et al. (2005).

Model 1 includes only additive genetic direct effects.

Model 2 includes additive genetic direct effects and maternal genetic effects.

For every model, the maternal genetic effect was nonsignificant ( $P > 0.05$ ).

no gene  $\times$  environment interaction in terms of how beak color responded to the environmental effect of exercise. We found no statistically significant environmental maternal effects for beak color (Table 3; see also Birkhead et al. 2005).

**Genetic variance in body condition.**—The coefficient of additive genetic variance (CVA) in body condition was estimated in the following way. Because our index of body condition was the residual from the log-log plot of tarsus mass (above), this did not allow us to calculate the CVA for body condition directly, we therefore standardized the residuals by adding the mean body mass to the residual from the untransformed tarsus versus body mass regression. This provided an estimate of  $CV_A = 5.70$  (Table 2). Using the logged values gave an estimate of 2.56. The genic capture hypothesis predicts relatively high additive genetic variance in condition, yet compared with some of the other parameters we measured (Table 2), neither of our estimates is especially high. However, it is not clear whether calculating  $CV_A$  in the way we have done here provides an estimate that is directly comparable with that of other traits (see Discussion). The high heritability estimate of condition ( $0.61 \pm 0.05$  SE,  $P < 0.001$ ; Tables 1, 2), suggests that much of the variation in condition is heritable. There was no maternal effect for body condition, or indeed for any other traits (Table 3). All three immune assays showed significant, but low heritabilities and moderate to high  $CV_A$  values (Tables 1, 2). Hematocrit showed high heritability and moderate  $CV_A$  (Tables 1, 2).

**Genetic correlation between condition and beak color.**—As predicted by the genic capture hypothesis, there was a significant positive genetic correlation between beak color and condition: beak before exercise = 0.387 ( $P < 0.01$ ); beak after exercise = 0.292 ( $P = 0.06$ ) (Table 2). The phenotypic correlations between beak color and condition were small, positive, and significant (beak before: 0.15,  $P < 0.001$ ; beak after: 0.13,  $P < 0.001$ ); the environmental correlations were smaller and nonsignificant (Table 2). Because evidence from other studies indicates that beak color in the zebra finch is condition dependent (Zann 1996; Blount et al. 2003), we predicted that it might decrease following the period of exercise, but this was not the case in the present study, suggesting that under some conditions zebra finches may maintain beak color at the expense of other traits. We obtained

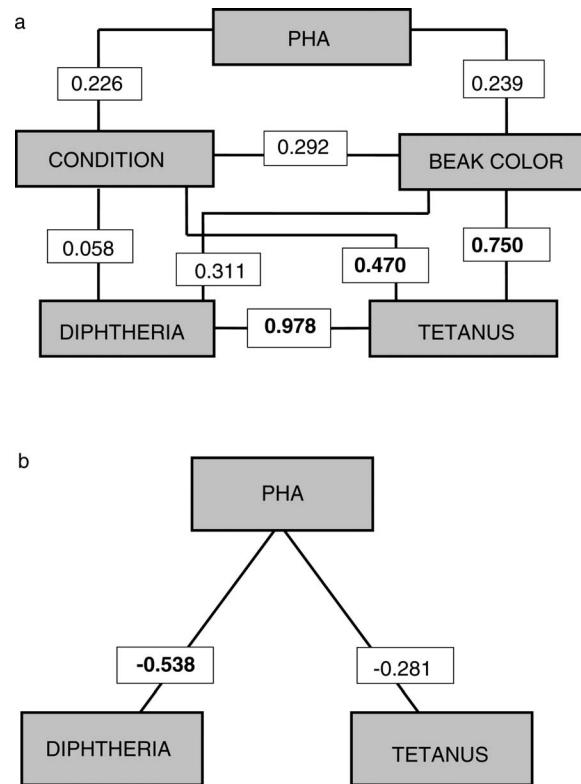


FIG. 1. (a) Genetic correlations between traits predicted to be positive by the genic capture hypothesis: all genetic correlations were positive and significant correlations ( $P < 0.05$ ) are indicated in bold. (b) Genetic correlations between different arms of the immune system; both are negative and the significant correlation ( $P < 0.05$ ) is indicated in bold.

similar results in a previous study (Birkhead et al. 1999) and together these indicate that maintaining this secondary sexual trait for mate acquisition may be important in the zebra finch, possibly because this is a very short-lived species (Zann 1996).

**Other genetic correlations.**—The genetic correlations between body condition and the immunological traits and between beak color and the immunological traits are shown in Figure 1a. The genic capture hypothesis predicts that these genetic correlations should all be positive, and although only three are statistically significant, that fact that eight of eight are positive is consistent with the hypothesis. A binomial test shows that eight of eight positive correlations is significant ( $P = 0.004$  one tailed, or  $P = (0.008$  two-tailed). Similarly, using Fisher's method of combining probabilities (Sokal and Rolf 1995) gives a  $\chi^2$  value of 48.19, 16 df,  $P < 0.001$ ; a more conservative analysis that exclude the high genetic correlation between the two humoral immune assays, is still significant ( $\chi^2 = 34.37$ , 14 df,  $P < 0.01$ ). A meta-analysis to test whether the mean genetic correlation differs significantly from zero is also significant (one-sample  $t$  test,  $t = 3.90$ , 7 df,  $P < 0.007$ ).

The individually statistically significant positive genetic correlations were those between beak color and tetanus, tetanus and condition, and between the two humoral immune assays (tetanus and diphtheria) (Fig. 1a). The genetic cor-

relations between the cell mediated immune assay (PHA) and the two humoral immune assays were both negative although only that between PHA and diphtheria was statistically significant (Fig. 1b; Table 2), indicating that trade-offs exist between different arms of the immune system (Cheng et al. 1991; Westneat and Birkhead 1998; Svensson et al. 1998; Cotter et al. 2004; Kilpimaa et al. 2005; Rolff et al. 2005).

#### DISCUSSION

The genic capture hypothesis states that variance in sexually selected traits is maintained by variance in condition and by a positive genetic correlation between the two (Iwasa and Pomiankowski 1994; Rowe and Houle 1996; Lorch et al. 2003; Tomkins et al. 2004). Our findings support some of the predictions of the genic capture hypothesis, specifically: (1) that genetic variance exists in condition, beak colour and immune function, and (2) that positive genetic correlations exist between some of these traits.

Our results relating to genetic correlations can be considered at two levels: (1) as a whole and (2) individually. As a whole the results are consistent with the genic capture hypothesis. Three of the eight predicted genetic correlations were significantly different from zero; all eight genetic correlations were positive, an effect that is significantly different from random, and in addition the mean genetic correlation was significantly different from zero.

Taken individually, the genetic correlations, some of which are statistically significant and some of which are not, provide mixed support for the genic capture hypothesis and are more difficult to interpret. It is well established that because of their large standard errors, genetic correlations require very large sample sizes to reach statistical significance (Falconer and Mackay 1996; Roff 1997; Lynch and Walsh 1998), and this may account for why only three of the eight predicted genetic correlations were formally statistically significant. The sample size in the present study (972 offspring from 81 sires and six dams per sire) was determined a priori by a power analysis in terms of obtaining heritability estimates (Falconer and Mackay 1996), not for detecting genetic correlations.

If we consider only those genetic correlations that are statistically significant (Fig. 1), our results do not support the genic capture hypothesis because no genetic covariance exists between condition and the sexually selected trait (beak color)—a key prediction of the genic capture hypothesis. However, our results do show convincingly that males in good condition and those with a red beak would exhibit a strong immune response to certain antigens (in this case, tetanus). Coltman et al. (2001) similarly found in feral sheep a positive genetic correlation between a sexually selected trait (body size) and parasite resistance. Indeed, because life-history traits tend to be condition dependent, substantial genetic variation in condition will tend to produce positive genetic correlations between traits (Reznick et al. 2000; Hunt et al. 2004). Our results indicate that in disease-free birds both an individual's genotype and its condition predict its ability to respond to a particular immune challenge.

Testing the genic capture hypothesis also depends on having a meaningful index of condition. Other studies that have

estimated the quantitative genetics of condition-dependent traits have either not manipulated body condition (e.g., Jensen et al. 2003; Gleeson et al. 2005), or have conducted much more drastic manipulations than we did, through diet manipulations (David et al. 2000) or starvation (Kotiaho et al. 2001). Each of these different approaches may be valid in their own circumstances, but they make comparisons between studies difficult.

The genic capture hypothesis predicts that there is substantial additive genetic variance in condition. However, for a variety of reasons (outlined below) it is difficult to decide what "substantial" means. To decide whether our estimate of  $CV_A$  for condition is "substantial" we can make comparisons between: (1) traits within our study and (2) estimates of  $CV_A$  for condition between studies. However, these comparisons assume that estimates are directly comparable. As we show, they are not. First, let us consider different traits within the present study. All the phenotypic traits in Table 2 except condition were measured directly, whereas condition was estimated very indirectly using residuals of log transformed values (see above). The combination of residuals and transformation introduces several problems, including scaling problems (Lynch and Walsh 1998) that preclude direct comparisons. This means that although residuals from a log-log regression of tarsus on body mass probably provide a biologically reasonable index of condition, the statistical properties of this index mean that it is of limited use in estimating  $CV_A$ . One of the problems with using residuals as an index of condition is that they have a mean of zero, making it difficult to calculate other genetic parameters such as  $CV_A$ . A solution to this is to add the mean body mass to each residual and base the calculation of  $CV_A$  on this, which is what we did. It is also what Kotiaho et al. (2001) did, although they do not state it in their paper (J. Kotiaho, pers. comm.). It is also what Merilä et al. (2001) did in their study (J. Merilä, pers. comm.). This makes comparisons between studies difficult, first because for organisms of different size it introduces a scaling effect (see below) and second, even for animals of similar size comparisons are valid only if exactly the same methodology had been employed. The utility of the coefficient of variation for comparing levels of variation between traits rests of the assumption that the standard deviation (SD) is directly proportional to the mean (Lynch and Walsh 1998: p. 301). If the raw data follow this pattern the  $CV_A$  is an appropriate measure of variation. However, in log transformed data the mean and SD become independent and the  $CV_A$  is no longer a valid measure of variability. There is a further difficulty: if one uses log transformed values (which solves the biological problem of a unit of resource being more valuable to a small individual than a large one (Kotiaho 1999)), an important scaling effect is introduced because the negative relationship between the mean and the  $CV_A$  results in small animals having larger  $CV_A$  values than larger animals, which further accounts for why the beetles in Kotiaho et al.'s (2001) study have higher  $CV_A$  values (see below) than the zebra finches in the present study. In this instance, a more valid measure of the variability is simply the variance of condition (calculated from the residuals of the logged relationship between size and mass).

Second, let us now compare estimates of  $CV_A$  between

studies. In fact there is only a single study with an estimate of the  $CV_A$  of condition that is even vaguely comparable with ours. As we have just seen, Kotiaho et al. (2001) used residuals from a log-log regression of body mass on size as an index of condition and added mean body mass to the residuals in order to calculate the  $CV_A$ . Their estimate of  $CV_A$  however was fairly substantial at 27.05. There are two possible reasons why their value is so much larger than ours. First, their experimental methodology differs from ours in that their manipulation of condition in the beetles via starvation was more extreme than our treatment, although it is not clear whether this would increase or decrease the variance in condition compared to the present study. More importantly, as just stated (above) their estimate of the  $CV_A$  of condition is inflated because of the scaling effect resulting from a combination of using log transformed values in the condition index and because their beetles are absolutely smaller than our zebra finches. Clear evidence that log transformation on its own inflates estimates of  $CV_A$  is apparent from a later study in which Simmons and Kotiaho (2002) used a subset of the data used in their earlier study (Kotiaho et al. 2001) to estimate  $CV_A$  from untransformed values and obtained an estimate of 8.14 (Simmons and Kotiaho 2002; J. Kotiaho, pers. comm.).

Comparison of the  $CV_A$  estimates between our two studies and between the traits within both our study and within Kotiaho et al.'s (2001) study are therefore difficult to interpret. In the present study all we can say is there is probably some additive genetic variance in condition, but whether it is substantial or not is unclear. Clearly, estimating condition in a manner that allows researchers to make meaningful comparisons between traits, between organisms, and between studies requires more thought and analysis. It is also important that researchers are aware that different methods provide very different estimates of  $CV_A$ .

It is also important to recognize that studies of animals in captivity may generate different estimates for genetic parameters from animals in nature. Views differ on the value of captive studies (Weigensberg and Roff 1996), but our pragmatic view is that a study in captivity is equivalent to looking at another population in a "different" environment. In addition, we consider that our study sets the stage for conducting comparable studies on wild birds.

In another study of the genetics of condition and immune function in the zebra finch, Gleeson et al. (2005) reported "substantial positive genetic covariance between immunocompetence and body condition" indicating a positive genetic correlation between condition and immune function measured as the PHA assay. However, their result is not directly comparable with ours for several reasons and hence—as Gleeson et al. (2005) themselves point out—should be treated with caution. First, their study was conducted on wild-caught zebra finches breeding in captivity, bred not as individual pairs but in large groups in aviaries with no check (e.g., using molecular techniques) on parentage before the genetic analyses were conducted. This may not be a serious problem because levels of extrapair paternity in the zebra finch are not high (Birkhead et al. 1988a, 1990), although intraspecific brood parasitism can be common, especially in captivity (Birkhead et al. 1990; Fenske and Burley 1996). Gleeson et al.'s (2005) index of body condition dif-

fered from ours in that theirs comprised residuals from an untransformed relationship between body mass and tarsus. Moreover, in contrast to our study, their measures of condition were made at two different ages: 17 days of age for young birds and as adults for their parents. In addition, Gleeson et al., (2005) did not manipulate condition in any way (e.g., through diet or exercise) and there was no validation of the body condition index. Further, their sample size was small, therefore the standard error associated with their proxy for a genetic correlation was large. Finally, in our study, although the genetic correlation between our index of condition and the PHA immune response was positive (Table 2), it was not statistically significant despite a much larger sample size. It would also have been interesting in the present context if Gleeson et al. (2005) had described the phenotypic and genetic relationships between beak color, condition, and immune function.

In conclusion, we seem to have raised more questions than we have answered. Despite the problems associated with estimating the  $CV_A$  of condition and the difficulty of rigorously testing the genic capture hypothesis, our results indicate the existence of genetic variation in body condition, and some significant positive genetic correlations between in condition, beak color and immune function.

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