Sex-specific heritability of cell-mediated immune response in the blue tit nestlings (*Cyanistes caeruleus*)

S. M. DROBNIAK*, D. WIEJACZKA*, A. ARCT*, A. DUBIEC†, L. GUSTAFSSON‡ & M. CICHOŃ*

*Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland
†Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland
‡Department of Animal Ecology, Evolutionary Biology Centre (EBC), Uppsala University, Uppsala, Sweden

Abstract

Here, we aimed at estimating sex-specific heritabilities of cell-mediated immune response (CMI) in the blue tit nestlings (*Cyanistes caeruleus*). To separate genetic and environmental components of the phenotypic variance in CMI (measured using phytohaemagglutinin assay), we performed a cross-fostering experiment. Additionally, controlled environmental variation was introduced by enlarging some broods. Our analyses revealed a significant genetic component (as approximated by the nest-of-origin term) of the phenotypic variance in immune response. More importantly, these genetic effects differed between sexes and experimentally manipulated brood sizes, as indicated by significant genotype-by-sex and genotype-by-environment interactions. We discuss possible causes of such sexual dimorphism in gene expression and suggest that sex- and environment-specific genetic interactions may contribute to the maintenance of genetic variability in traits related to immune functions.

Introduction

Parasite-imposed selective pressures are thought to play an important role in the array of evolutionary processes, such as the evolution of sexual reproduction and sexual selection (Hamilton, 1980), speciation (Goater & Holmes, 1997) and the evolution of life histories (Stearns, 1992). The most spectacular evolutionary product of selective pressure of pathogens is immune system (Wakelin & Apanius, 1997). Importance of immune function is particularly apparent among individuals suffering from immunodeficiency syndromes (such as severe combined immunodeficiency disease or AIDS); such individuals cannot fight any illness and eventually die because of various infections caused by microorganisms being otherwise opportunistic (Roitt & Delves, 2006). However, over reactive immune system may lead to autoimmune diseases. Thus, the immune function constitutes an important selective target. There is a growing

evidence for a direct link between immunity and fitness, mainly in the form of positive correlations between immunocompetence and some proxies of fitness (survival, recruitment to reproduction; Gonzalez *et al.*, 1999; Møller & Saino, 2004; Cichoń & Dubiec, 2005). However, fitness-related traits can exhibit significant evolutionary response to selection only if there is a significant genetic component in their phenotypic variance (Rice, 2004).

Evidence for significant heritabilities in immune-related traits is equivocal. Artificial-selection experiments for high and low immune response in the chicken *Gallus gallus* yielded estimates of heritability ranging from 0.12 to 0.48 (for cellular response) and from 0.19 to 0.60 (for Ig titres) (Cheng *et al.*, 1991; Kean *et al.*, 1994; Sarker *et al.*, 1999). Some studies performed on wild bird populations report significant heritabilities of parasite resistance or immune response (Saino *et al.*, 1997; Brinkhof, 1999; Soler *et al.*, 2003; Ardia, 2005; Ardia & Rice, 2006; Cichoń *et al.*, 2006), whereas others failed to show significant genetic component of immune function (Christe *et al.*, 2000; Tella *et al.*, 2000; De Neve *et al.*, 2004; Kilpimaa *et al.*, 2005; Pitala *et al.*, 2007). However, gene expression may vary across environments, which

Correspondence: Szymon Drobniak, Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland.
Tel.: +48 012 664 52 19; fax: +48 012 664 69 12;
e-mail: szymek.drobniak@uj.edu.pl

might lead to nonsignificant estimates of heritability. Such genotype-by-environment interaction (GEI) has been observed both in morphological and in life-history traits in birds (Merilä & Fry, 1998; Charmantier *et al.*, 2004; De Neve *et al.*, 2004; Garant *et al.*, 2004; Pitala, 2007). GEI can be expressed in two (not mutually exclusive) forms: (i) environments may differ in expression of genetic variance, i.e. heritabilities may differ between environments; (ii) reaction norms (phenotypic functions of environmental conditions) may cross within the range of possible environmental conditions (Lynch & Walsh, 1998). The crossing of reaction norms is particularly interesting because it is believed to have potential of maintaining variability in traits subjected to strong directional selection (Lynch & Walsh, 1998). Such variability in reaction norms is usually expected when selection favours different alleles in different environments (Lazzaro *et al.*, 2008).

Interestingly, variability in gene expression may be driven by other factors, not only environmental. Existing evidence suggests that expression of genetic variance may also differ between sexes. Jensen *et al.* (2003) reported sex-specific heritabilities of several morphological traits in house sparrows (*Passer domesticus*). A meta-analysis on studies reporting heritabilities of morphological traits in birds unambiguously shows higher heritabilities in females (Jensen *et al.*, 2003). Such sexual differences in immunity-related traits might also be expected. There is a growing evidence that sexes may respond differently to changes in environmental conditions (e.g. Badyaev *et al.*, 2001; Rutkowska & Cichoń, 2005; Dubiec *et al.*, 2006). Such sexual dimorphism in environmental sensitivity may result from different proportions of environmental and genetic variance in the overall phenotypic variance. Consequences of observed dimorphism in the genetic architecture of males and females might be numerous. It is believed to play an important role both in the evolution of sexual dimorphism (Potti & Merino, 1996; Badyaev, 2002) and in determining the rate of evolutionary change in a population (Jensen *et al.*, 2003).

Here, we estimate sex-specific heritabilities and cross-sex genetic correlations of cell-mediated immune response (CMI) in the blue tit (*Cyanistes caeruleus*) nestlings. CMI was measured by phytohaemagglutinin (PHA) hypersensitivity assay, which is considered to be a reliable measure of this type of immunity (Goto *et al.*, 1978; Lochmiller *et al.*, 1993; Smits *et al.*, 1999; Tella *et al.*, 2008; but see also Martin *et al.*, 2006). By incorporating experimentally controlled environmental variation (manipulation of brood size simulating good and poor rearing conditions), we also searched for indications of GEI. This was accompanied by cross-fostering – a commonly used experimental technique that, together with proper statistical approach (linear mixed modelling), allows for breaking down causal components of phenotypic variance into its genetic and environmental parts (Merilä, 1996, 1997; Merilä & Fry, 1998). Based on previous studies, we predict that heritability of CMI is higher in females compared to that in males.

Materials and methods

Study system and general methods

The study was conducted in 2007, 2008 and 2009 in the population of blue tits breeding in nest-boxes on the Swedish island of Gotland (57°03′N, 18°17′E). The study area consists of lowlands covered by deciduous and mixed-coniferous forests separated by cultivated fields and hay-meadows (see Pärt & Gustafsson (1989) for more detailed description of the study area). In this population, blue tits lay one or occasionally two clutches per season. Females lay on average 10–13 eggs. Young hatch after 2 weeks of incubation provided exclusively by a female and fledge after 18–22 days post-hatching. Relatively large clutches make blue tits particularly suitable for performing manipulations even in periods of increased nestling mortality.

From the end of April, we regularly inspected nest-boxes to find all blue tit clutches. For each brood, the number of eggs, the date of laying and the date of hatching (day 0) were recorded. Nestlings were uniquely marked by nail clipping (day 2) and ringing (day 11). All nestlings were measured for tarsus length (day 14; with electronic calliper to the nearest 0.01 mm) and weighed (day 2, 11, 12, 14; with Pesola spring balance to the nearest 0.1 g).

Experimental procedures

Broods hatched at the same date and of similar size (±1 nestling) were paired and cross-fostered by exchanging half of the brood between the nests. Additionally, one randomly selected brood in each pair was enlarged by adding three nestlings from a donor nests (not included in the analyses). Donor nestlings were chosen to have intermediate weight.

In total, 59 pairs were created. Eight broods failed to survive to fledging, and thus eight pairs of broods consisting of these unsuccessful nests were excluded from final analyses. As the sex-ratio could potentially influence within-nest competition (and bias selective pressure with respect to one of the sexes), brood sex composition in control and enlarged broods was compared on day 2 post-hatching. No significant differences were observed (generalized linear model using a logit link function and binomial distribution, $\chi^2_1 = 0.61$, $P = 0.43$). Another possible source of bias in estimates of heritability would be misassigned paternities because of differences in sex-ratio between within- and extra-pair young. However, Kempenaers *et al.* (1997) showed that paternity does not affect sex-ratios in the blue tits. As

Charmantier & Réale (2005) showed, the bias of h^2 estimates because of misassigned paternity should not be > 15%; thus, we assume it should not alter significantly our qualitative inferences.

Cell-mediated immunity measurements

CMI is the type of immunity that relies on activation of various types of immunoactive cells (e.g. macrophages, NK cells, cytotoxic T lymphocytes) rather than antibodies or complement. Here, CMI was measured with a PHA assay. PHA is a plant-derived lectin that has a strong mitogenic effect on T lymphocytes, serving as a model nonpathogenic antigen (Goto *et al.*, 1978). In our experiment, 0.2 mg of PHA (Sigma Aldrich, Poznan, Poland) suspended in 0.04 mL of saline was injected to the right wing web. The thickness of the wing web was measured thrice prior to and 24 h after the injection using pressure-sensitive spessimeter (Mitutoyo, Tokyo, Japan). All measurements were taken by the same person and were highly repeatable (prior to injection: $r = 0.97$, $F_{580,1162} = 73.90$, $P < 0.0001$; after injection: $r = 0.99$, $F_{580,1162} = 136.25$, $P < 0.0001$; Lessells & Boag, 1987). Mean value of three repeats was used in further analyses. The level of immune response was expressed as the intensity of swelling and was calculated as the difference between wing-web thickness prior to and after the injection (Smits *et al.*, 1999).

Molecular sexing

All nestlings were blood-sampled on the second day post-hatching. Blood was drawn from the leg vein to the capillary and stored at room temperature in 96% ethanol until analysed. Whole-blood DNA was extracted using Chelex (Bio-Rad, Munich, Germany) (Walsh *et al.*, 1991; see Cichoń *et al.*, 2003 for further details). Sexing was performed following protocol of Griffiths *et al.* (1998). This is a PCR-based technique that involves amplification of homologous fragments of chromohelicase (CHD) gene located on both Z and W sex chromosomes.

Statistical analyses

Phenotypic variance of any trait contains components attributable both to heritable genetic effects and to environmental factors. In this study, genetic effects are represented by nest-of-origin, whereas experimental block (pair of nests), residual variance and nest-of-rearing constitute environmental variance. All of these are defined as random effects and are of key interest in our study. To partition genetic and environmental variance, general linear mixed models were applied. All models had similar structure, fitting fixed effects (sex, year and experimental group) and an appropriate set of random effects. The compositions of the final models were decided upon the *deviance information criterion* (*DIC*)

values. In the final analyses, we used a series of three successive models, listed below:

(A) CMI = fixed effects + nest-of-origin + nest-of-rearing + block + residual – this univariate model allowed for estimation of overall broad-sense heritability.

(B) bivariate formulation of the above with CMI in each sex used as a separate trait – this allowed for estimation of cross-sex genetic correlations and sex-specific genetic variances.

(C) bivariate formulation of the first model with CMI in each experimental treatment modelled as a separate trait – estimation of cross-environment genetic correlations and environment-specific genetic variances.

Calculation of the proportion of variance explained by nest-of-origin effect in overall phenotypic variance allowed for calculation of broad-sense heritabilities. In our analysis, we assume that all individuals in one nest of origin are full-siblings and that there is no relatedness between families. In such a case nest-of-origin effect approximates half of additive genetic variance plus quarter of dominance variance and maternal effects (ME) if present (Lynch & Walsh, 1998). The denominator in heritability estimates was the sum of all variances estimated by the model (nest of origin + nest of rearing + residual).

All variance/covariance estimates and genetic correlations were obtained with Markov Chain Monte Carlo method using R 2.9.2 and *MCMCglmm* package (Hadfield, 2010). Univariate and bivariate models were fitted with the following parameters, respectively: number of iterations – 60 000 and 130 000; burn-in period – 7000 and 30 000; thinning interval – 50 and 100. Time-series plots showed no autocorrelation issues. Priors for univariate analyses were defined using overall phenotypic variance (V_P) partitioned in the following way: nest-of-origin – $0.2 \times V_P$; nest-of-rearing – $0.1 \times V_P$; block – $0.1 \times V_P$; residual – $0.6 \times V_P$. The information content parameter (n) was set to 1. For bivariate models, priors were specified similarly except that V_P was a 2×2 matrix containing respective variances and covariance equal to 0, with $n = 2$. To check the influence of different priors, additional models were fitted using priors with V_P partitioned equally between random effects; these models yielded results that were slightly different from the previous ones – but quantitative and qualitative conclusions remained identical. DIC was used to select the most appropriate set of random effects (i.e. the best-fitting model; Table S2). Significance of genetic correlations being different from one was assessed using confidence intervals (CI). 95% CI for all (co)variances and genetic correlations were calculated from estimates' posterior distributions using highest-posterior-density function (*HPDinterval*, library *coda*). Because each individual was measured for only one sex/environment-specific trait, all random effect covariances in multivariate models were fixed at zero, except the genetic ones (nest-of-origin effects).

Table 1 Estimates of variance components of the cell-mediated immunity from the univariate model (A). Variance estimates and heritability are listed, together with their respective 95% confidence intervals.

Effect	Variance	Var. 95% CI	H^2	H^2 95% CI
Nest of origin	0.0100	(0.0062;0.0149)	0.38	(0.16;0.51)
Nest of rearing	0.0113	(0.0054;0.0178)		
Block	0.0095	(0.0027;0.0191)		
Residual	0.0207	(0.0185;0.0243)		

Results

The results of all analyses are listed in Tables 1 and 2 and in the supplementary online materials. In the univariate model (A), all variance estimates appeared significantly different from zero, as presented using 95% CIs (Table 1) and supported by DIC-based selection of best-fitting model (Table S2). Nest of origin effect explained 19.5% of phenotypic variance in CMI, which results in broad-sense heritability estimate of 0.39.

In both the bivariate models (B and C), all variance estimates were significantly different from zero (see Table S1). For genetic effects (as estimated using nest-of-origin factor), we also obtained estimates of cross-sex and cross-environment covariances and correlations (Table 2). Both between sexes and between experimental treatments genetic correlations of CMI are low and significantly different from one. Additionally, cross-sex genetic correlation is not significantly different from zero. This indicates significant crossing of reaction norms both between sexes and between environments (experimental treatments). Heritability of CMI among females tended to be higher compared to heritability among males [respective H^2 (95% CI) for females: 0.64 (0.48; 0.87) and males: 0.07 (0; 0.56)]. In case of environment-specific heritabilities, large overlap between their respective confidence intervals was observed [H^2 (95% CI) for control: 0.43 (0.26; 0.68) and experimental group: 0.36 (0.14; 0.53)], suggesting no significant differences.

Table 2 Genetic variance and covariance estimates from the bivariate models (B, C) for environment- and sex-specific cell-mediated immunity. Diagonals contain sex/environment-specific variance estimates with 95% CI, whereas off-diagonal elements contain covariance with 95% CI (above diagonal) and cross-sex/environment genetic correlations with 95% CI (below diagonal).

	Males	Females
Males	0.0015 (0;0.0175)	0.0014 (−0.0027;0.0106)
Females	r_g = 0.2632 (−0.1007;0.7232)	0.0189 (0.0073;0.0328)
	Enlarged	Control
Enlarged	0.0096 (0.0051;0.0169)	0.0028 (−0.0021;0.0092)
Control	r_g = 0.2910 (0.1090;0.5811)	0.0104 (0.0059;0.0183)

Discussion

Here, we report evidence for significant genetic component of phenotypic variance in CMI in blue tit nestlings. However, the expression of the genetic variance appeared complex. Genes for CMI seem to be differently expressed across environments and also between sexes. A significant genetic component of variation in PHA response has been reported in several studies (Brinkhof, 1999; Tella *et al.*, 2000; De Neve *et al.*, 2004; Ardia, 2005; Ardia & Rice, 2006; Cichoń *et al.*, 2006). Sex-specific genetic effects underlying immune response were investigated only in invertebrates (mealworm beetle *Tenebrio molitor*, Rolff *et al.*, 2005). This study showed higher heritability of hemocyte titres (directly related to immune response in insects) among males. Our study is the first to show sex-specific genetic architecture of immune response in vertebrates. Sex-specific genetic effects have already been reported for morphological traits in birds (with females exhibiting higher heritabilities; Jensen *et al.*, 2003). Our study indicates that sex-specific genetic architecture of life-history related traits might be more complex than for morphological traits, as reflected by low genetic correlations. Genetic correlations were significantly less than one both between sexual group and between experimental treatments, indicating low origin-specific cross-sex/environment correlation and existence of significant genotype-by-environment and genotype-by-sex interactions.

Consequences of genetic interactions observed here are numerous. There is growing evidence that the level of immune response might be a subject of natural selection (Råberg & Stjernman, 2003; Cichoń & Dubiec, 2005). Cichoń & Dubiec (2005) reported directional selective pressure acting on cell-mediated immunity in this particular population of blue tits. In such a case, evolutionary theory predicts erosion of additive genetic variance and decrease of heritability (Lynch & Walsh, 1998). Many studies, however, show genetic components in immunity-related traits to be high and statistically significant (Saino *et al.*, 1997; Brinkhof, 1999; Soler *et al.*, 2003; Ardia, 2005; Cichoń *et al.*, 2006). Thus, some mechanism maintaining this variability must exist. Interaction between genetic properties and environment are generally believed to be one of possible mechanisms of maintenance of genetic variability. One type of GEI – i.e. one in which reaction norms cross within the range of possible environmental conditions – attracts most of the research interest. If reaction norms cross, genotypes coding for low and high fitness are differently ranked in distinct environments. This creates potential of maintaining genetic variability by partial decomposition of bonds between genotype and phenotype throughout the environmental continuum. GEI in immunocompetence-related traits has been reported in several taxa (De Neve *et al.*, 2004; Ojala, 2005; Pitala, 2007; Lazzaro *et al.*, 2008; Williams *et al.*, 2008). Types of environmental gradients

and immunological functions of interest vary across these studies but in general they suggest that GEIs associated with immunocompetence might be common. In the current study, we report genetic components of CMI to differ in their ranking across different environments. Thus, the observed interactions may be proposed as one of possible mechanisms maintaining genetic variability in immune functions in our population.

Genotype-by-sex interaction can be considered as a very special type of GEI, and thus it also might play a role in maintenance of genetic variability in immunity-related traits. An intriguing question is, however, on the origin of sexual differences in expression of genetic components. There might be several, not necessarily exclusive, explanations:

(1) Sex-specific expression of genes: this could be mediated by sexual hormones or other endocrine factors acting differentially in males and females. For example, steroid hormones have been shown to affect immune function in several studies (Yao *et al.*, 2003; Muehlenbein & Bribiescas, 2005) but clear experimental evidence is scarce (Nunn *et al.*, 2009). These factors can indeed have a profound effect on genetic expression because many of them are associated with transcriptional factors.

(2) Sex-specific selective bias: males and females have been reported to differ with respect to their sensitivity to unfavourable environmental conditions (Potti & Merino, 1996; Badyaev *et al.*, 2001; Rutkowska & Cichoń, 2005; Dubiec *et al.*, 2006). Sexual size dimorphism and pressures imposed by sexual selection generate physiological trade-offs mediating these differences in sex-specific environmental sensitivity (see Dubiec *et al.*, 2006 for more details). Consequently, males would experience stronger selective pressures in comparison with females resulting in erosion of additive genetic variance and the observed sex-specific bias in heritabilities.

(3) Sex-specific ME: females deposit in eggs various substances influencing development of their offspring (e.g. hormones, antibodies, antioxidants). If mothers differentiated their investment in male and female eggs, this would influence sex-specific estimates of broad-sense heritability (because ME are inseparable in the cross-fostering method). Although interference of ME cannot be entirely ruled out most studies assume that their influence is negligible (Kruuk *et al.*, 2001; Kilpimaa *et al.*, 2005).

Irrespectively of its origin, sexual bias in heritabilities might significantly influence evolutionary processes acting at the level of population. Because of higher heritability, females should respond stronger to natural selection. Thus, the rate of evolutionary change in a trait with such a sexually biased genetic architecture would be determined mainly by response to selection in one of the sexes. If so, any processes changing sex-ratio in the population should also alter rate of evolutionary change. This is especially intriguing given birds' abilities to manipulate sex-ratio of their offspring (Komdeur *et al.*, 1997; Sheldon *et al.*, 1999; Pryke & Griffith, 2009; see also Rutkowska & Badyaev, 2009 for a review).

To conclude, our study suggests that genetic architectures of males and females can significantly differ. It emphasizes the need of separate analyses of quantitative traits within sexes. This approach can give important insights into the complexity of processes shaping the evolution of quantitative traits. Neglecting sexual differences in heritabilities could also make the outcomes of artificial-selection programs less predictable. Finally, the interplay between sex, environmental conditions and genetic effects gives new insight into mechanisms regulating level of genetic variance in immune function, with G × E and G × Sex interactions reported in this study being the most probable candidate factors maintaining genetic variance in traits subjected to strong sexual selection.

Acknowledgments

References

Ardia, D. 2005. Cross-fostering reveals an effect of spleen size and nest temperatures on immune responses in nestling European starlings. *Oecologia* **145**: 326–333.

Ardia, D. & Rice, E. 2006. Variation in heritability of immune function in the tree swallow. *Evol. Ecol.* **20**: 491–500.

Badyaev, A.V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* **17**: 369–378.

Badyaev, A., Whittingham, L. & Hill, G. 2001. The evolution of sexual size dimorphism in the House Finch. III. Developmental basis. *Evolution* **55**: 176–189.

Brinkhof, M.W.G. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc. R. Soc. Lond. B Biol. Sci.* **266**: 2315–2322.

Charmantier, A. & Réale, D. 2005. How do misassigned paternities affect the estimation of heritability in the wild? *Mol. Ecol.* **14**: 2839–2850.

Charmantier, A., Kruuk, L.E.B. & Lambrechts, M.M. 2004. Parasitism reduces the potential for evolution in a wild bird population. *Evolution* **58**: 203–206.

Cheng, S., Rothschild, M.F. & Lamont, S.J. 1991. Estimates of quantitative genetic parameters of immunological traits in the chicken. *Poult. Sci.* **70**: 2023–2027.

Christe, P., Moller, A.P., Saino, N. & De Lope, F. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). *Heredity* **85**(Pt 1): 75–83.

Cichoń, M. & Dubiec, A. 2005. Cell-mediated immunity predicts the probability of local recruitment in nestling blue tits. *J. Evol. Biol.* **18**: 962–966.

Cichoń, M., Dubiec, A. & Stoczko, M. 2003. Laying order and offspring sex in blue tits *Parus caeruleus*. *J. Avian Biol.* **34**: 355–359.

Cichoń, M., Sendecka, J. & Gustafsson, L. 2006. Genetic and environmental variation in immune response of collared flycatcher nestlings. *J. Evol. Biol.* **19**: 1701–1706.

De Neve, L., Soler, J.J., Perez-Contreras, T. & Soler, M. 2004. Genetic, environmental and effects on magpie nestling-fitness traits under different nutritional conditions: a new experimental approach. *Evol. Ecol. Res.* **6**: 415–431.

Dubiec, A., Cichoń, M. & Deptuch, K. 2006. Sex-specific development of cell-mediated immunity under experimentally altered rearing conditions in blue tit nestlings. *Proc. R. Soc. Lond. B Biol. Sci.* **273**: 1759–1764.

Garant, D., Kruuk, L.E.B., McCleery, R.H. & Sheldon, B.C. 2004. Evolution in a changing environment: a case study with great tit fledging mass. *Am. Nat.* **164**: E115–E129.

Goater, C.P. & Holmes, J.C. 1997. Parasite-mediated natural selection. In: *Host–Parasite Evolution: General Principles and Avian Models* (D.H. Clayton & J. Moore, eds), pp. 9–29. Oxford University Press, Oxford.

Gonzalez, G., Sorci, G. & de Lope, F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* **46**: 117–122.

Goto, N., Kodama, H., Okada, K. & Fujimoto, Y. 1978. Suppression of phytohemagglutinin skin response in thymectomized chickens. *Poult. Sci.* **57**: 246–250.

Griffiths, R., Double, M., Orr, K. & Dawson, R. 1998. A DNA test to sex most birds. *Mol. Ecol.* **7**: 1071–1075.

Hadfield, J. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1–22.

Hamilton, W.D. 1980. Sex versus non-sex versus parasite. *Oikos* **35**: 282–290.

Jensen, H., Sæther, B., Ringsby, T.H., Tufto, J., Griffith, S.C. & Ellegren, H. 2003. Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (*Passer domesticus*). *J. Evol. Biol.* **16**: 1296–1307.

Kean, R.P., Cahaner, A., Freeman, A.E. & Lamont, S.J. 1994. Direct and correlated responses to multitrait, divergent selection for immunocompetence. *Poult. Sci.* **73**: 18–32.

Kempenaers, B., Verheyen, G. & Dhondt, A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behav. Ecol.* **8**: 481–492.

Kilpimaa, J., Van de Casteele, T., Jokinen, I., Mappes, J. & Alatalo, R.V. 2005. Genetic and environmental variation in antibody and T-cell mediated responses in the great tit. *Evolution* **59**: 2483–2489.

Komdeur, J., Daan, S., Tinbergen, J. & Mateman, C. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. *Nature* **385**: 522–525.

Kruuk, L., Merilä, J. & Sheldon, B. 2001. Phenotypic selection on a heritable size trait revisited. *Am. Nat.* **158**: 557–571.

Lazzaro, B., Flores, H., Lorigan, J. & Yourth, C. 2008. Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. *PLoS Pathog.* **4**: e1000025.

Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116–121.

Lochmiller, R., Vestey, M. & Boren, J. 1993. Relationship between protein nutritional status and immunocompetence in northern Bobwhite chicks. *Auk* **110**: 503–510.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland.

Martin, L., Han, P., Lewittes, J., Kuhlman, J.R., Klasing, K.C. & Wikelski, M. 2006. Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoecological technique. *Funct. Ecol.* **20**: 290–299.

Merilä, J. 1996. Genetic variation in offspring condition: an experiment. *Funct. Ecol.* **10**: 465–474.

Merilä, J. 1997. Expression of variation in body size of collared flycatcher under different environmental conditions. *Evolution* **51**: 526–536.

Merilä, J. & Fry, J.D. 1998. Genetic variation and causes of genotype–environment interaction in the body size of Blue Tit (*Parus caeruleus*). *Genetics* **148**: 1233–1244.

Møller, A.P. & Saino, N. 2004. Immune response and survival. *Oikos* **104**: 299–304.

Muehlenbein, M. & Bribiescas, R. 2005. Testosterone-mediated immune functions and male life histories. *Am. J. Hum. Biol.* **17**: 527–558.

Nunn, C., Lindenfors, P., Pursall, E. & Rolff, J. 2009. On sexual dimorphism in immune function. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**: 61–69.

Ojala, K. 2005. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. *Evol. Ecol. Res.* **7**: 1153–1170.

Pärt, Y. & Gustafsson, L. 1989. Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): possible causes and reproductive consequences. *J. Anim. Ecol.* **58**: 305–320.

Pitala, N. 2007. *Causes and Consequences of Variation in Nestling Immune Function*. PhD Thesis, Helsinki, Finnland.

Pitala, N., Gustafsson, L., Sendecka, J. & Brommer, J.E. 2007. Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers. *Biol. Lett.* **3**: 418–421.

Potti, J. & Merino, S. 1996. Parasites and the ontogeny of sexual size dimorphism in a Passerine bird. *Proc. R. Soc. Lond. B Biol. Sci.* **263**: 9–12.

Pryke, S.R. & Griffith, S.C. 2009. Genetic incompatibility drives sex allocation and maternal investment in a polymorphic finch. *Science* **323**: 1605–1607.

Råberg, L. & Stjernman, M. 2003. Natural selection on immune responsiveness in Blue Tits *Parus caeruleus*. *Evolution* **57**: 1670–1678.

Rice, S.H. 2004. *Evolutionary Theory: Mathematical and Conceptual Foundations*. Sinauer Associates, Sunderland.

Roitt, I.M. & Delves, P.J. 2006. *Roitt's Essential Immunology*. Blackwell Publishing, London.

Rolff, J., Armitage, S.A.O. & Coltman, D.W. 2005. Genetic constraints and sexual dimorphism in immune defense. *Evolution* **59**: 1844–1850.

Rutkowska, J. & Badyaev, A.V. 2009. Meiotic drive and sex determination: molecular and cytological mechanisms of sex ratio adjustment in birds. *Proc. R. Soc. B. Lond. Biol. Sci.* **363**: 1675–1686.

Rutkowska, J. & Cichoń, M. 2005. Egg size, offspring sex and hatching asynchrony in zebra finches *Taeniopygia guttata*. *J. Avian Biol.* **36**: 12–17.

Saino, N., Calza, S. & Moller, A.P. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* **66**: 827–836.

Sarker, N., Tsudzuki, M., Nishibori, M. & Yamamoto, Y. 1999. Direct and correlated response to divergent selection for serum immunoglobulin M and G levels in chickens. *Poult. Sci.* **78**: 1–7.

Sheldon, B., Andersson, S., Griffith, S., Örnborg, J. & Sendecka, J. 1999. Ultraviolet colour variation influences blue tit sex ratios. *Nature* **402**: 874–877.

Smits, J.E., Bortolotti, G.R. & Tella, J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**: 567–572.

Soler, J.J., Moreno, J. & Potti, J. 2003. Environmental, genetic and maternal components of immunocompetence of nestling pied flycatchers from a cross-fostering study. *Evol. Ecol. Res.* **5**: 259–272.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

Tella, J.L., Bortolotti, G.R., Forero, M.G. & Dawson, R.D. 2000. Environmental and genetic variation in T-cell-mediated immune response of fledgling American kestrels. *Oecologia* **123**: 453–459.

Tella, J.L., Lemus, J.A., Carrete, M. & Blanco, G. 2008. The PHA test reflects acquired T-cell mediated immunocompetence in birds. *PLoS ONE* **3**: e3295.

Wakelin, D. & Apanius, V. 1997. Immune defence: genetic control. In: *Host–Parasite Evolution: General Principles and Avian Models* (D.H. Clayton & J. Moore, eds), pp. 30–58. Oxford University Press, Oxford.

Walsh, P., Metzger, D. & Higuchi, R. 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**: 506–513.

Williams, L., Oliver, J., Peterson, E., Bobbitt, K., McCabe, M. Jr, Smolarek, D., Havstad, S., Wagienka, G., Burchard, E., Ownby, D. & Johnson, C. 2008. Gene-environment interactions between CD14 C-260T and endotoxin exposure on Foxp3 + and Foxp3- CD4 + lymphocyte numbers and total serum IgE levels in early childhood. *Ann. Allergy Asthma Immunol.* **100**: 128–136.

Yao, G., Liang, J., Han, X. & Hou, Y. 2003. In vivo modulation of the circulating lymphocyte subsets and monocytes by androgen. *Int. J. Immunopharmacol.* **3**: 1853–1860.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Summary of all models fitted to the data.

Table S2 Model selection based on DIC values.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.