

## Variation in heritability of immune function in the tree swallow

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**Abstract** Heritability is an important component of the ability of a trait to respond to natural selection; variation in heritability can lead to differences in how a trait responds to selection pressures. Here we test whether an important physiological trait, immune function, varies by comparing heritability estimates through cross-fostering brood manipulation at three widespread sites in the tree swallow (*Tachycineta bicolor*): Alaska, New York and Tennessee. In two of three sites, there was no additive genetic component to nestling immune response to the mitogen phytohaemagglutinin, while immune response had a heritable component in Tennessee. Bootstrapping revealed significant differences in estimated heritability. This conclusion was supported by mother–offspring regressions; in Tennessee breeding females mounting strong immune responses tended to have offspring with strong immune responses, while in New York and Alaska, there was no relationship between the immune responses of mothers and offspring. These results suggest that studies investigating the roles of common origin and rearing environment should consider yearly or spatial variation within a species.

**Keywords** Heritability · Immune function · Environmental variation · Cross-fostering · *Tachycineta*

### Introduction

The ability of a trait to respond to natural selection depends in part on the narrow-sense heritability, the ratio of additive genetic variance to total phenotypic variance (hereafter

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“heritability”). Low heritability may arise because additive genetic variance is low, because environmental variance is high, or because of some combination of the two (Falconer and Mackay 1996). Recent studies have found that the exposure of traits to selection can vary with environmental conditions due to differences in trait heritability, suggesting that the rate of evolutionary change can vary among populations (Mousseau and Roff 1987; Houle 1992; Roff et al. 2002; Charmanier and Garant 2005). Therefore, it is useful, from an evolutionary standpoint, to assess the relative contribution of both genetic and environmental components of phenotypic variance among populations and over time predict whether a single time point accurately reflects heritability over time.

In this study we assessed variation in heritability in a trait important to fitness: a component of immune function. In birds, the strength and responsiveness of the immune system is a predictor of return rates and yearly survival in birds (Hörak et al. 1999; Christe et al. 2001; Ardia et al. 2003; Møller and Saino 2004) and is important because of tradeoffs between immune function and other life history traits (Martin et al. 2001; Tella et al. 2002). The ability to mount a strong immune response has a strong genetic component, shown, for example, in the important role of major histocompatibility complex (MHC) genes in the cellular immune response (Taylor et al. 1987; Warner et al. 1987; Cheng et al. 1991), indicating that immune function has the potential to respond to natural selection, results supported in a field study in blue tits (*Parus caeruleus*) (Raberg and Stjernman 2003). Interestingly, the influence of additive genetic variance (i.e. genetic variance associated with the average effects of substituting one allele for another) on heritability of immune response varies depending on the strength of selection (Nath et al. 2001; Zekarias et al. 2002; Davison 2003) suggesting that a single assessment may not accurately reflect heritability across a species.

Here we take advantage of a cross-fostering brood manipulation conducted as part of a larger study (Ardia 2005a, 2005b) to compare genetic and environmental components of immune function at three widely-separated sites across the range of the tree swallow (*Tachycineta bicolor* Vieillot), a cavity-nesting North American passerine bird. Our primary goal was to determine whether heritability differs among sites or years in the tree swallow. We chose to analyze the relative contributions of common origin and rearing environment *within each* site rather than grouping sites; this allowed us to assess the heritability of immune function within the context of environmental and genetic variation at each site, rather than through the artificial pooling of variance among sites. The sites examined span the breeding range of the tree swallow and differ in food availability, environmental conditions and life history tradeoffs (Ardia 2005a, 2005b). Because we have only one year of data per site, we were unable to separate the effects of yearly variation versus geographic variation. However, our data set does allow us to test whether heritability remains similar over time or space in order to assess whether heritability remains constant in this species.

Cross-fostering allowed us to partly determine the relative role of rearing environment and common nest origin by comparing immune function of related offspring when raised in the natal nest versus out-reared (Saino et al. 1997; Brinkhof et al. 1999; Tella et al. 2000; de Neve et al. 2004). This design created a genotype-by-environment interaction reflected in whether the role of common nest origin would vary among experimental brood sizes. Overall, we sought to determine whether heritability varied in the tree swallow (either

among populations or over time) in order to examine the underlying ability of immune function to respond to natural selection.

## Materials and methods

Tree swallows (*Tachycineta bicolor* Vieillot 1808) are common cavity-nesting migrant birds in the family Hirundinidae that breed across North America (Robertson et al. 1992). Tree swallows breeding in nestboxes erected for their use were studied from May to July 2000 in Tompkins County, New York, USA (centered around 42°29' N, 76°27' W); from April to July 2001 in Loudon, Anderson, and Knox Counties, Tennessee, USA (centered around 35°53' N, 84°18' W); and from May to July in 2002 near Fairbanks, Alaska, USA (centered around 64°49' N, 147°52' W). Timing of breeding varied among sites (median date: Tennessee 21 April, New York 10 May, Alaska 1 June); the experiment was conducted on nests spanning 82–85% of the breeding season at each site.

Nests were checked daily to determine clutch size and date of clutch initiation. First broods with identical hatching dates were assigned randomly to one of three clutch size treatments: (1) reduced, (2) control, or (3) enlarged to create broods that were roughly 50% smaller or larger than original clutch size (avg. number of nestlings on day four; Alaska: reduced 2.94,  $N=16$ , control 5.18,  $N=17$ , enlarged 7.88,  $N=16$ ; New York: reduced 3.00,  $N=18$ , control 5.22,  $N=18$ , enlarged 7.88,  $N=17$ ; Tennessee reduced 2.94,  $N=18$ , control, 5.00,  $N=18$ , enlarged 7.72,  $N=18$ ; total number of chicks moved among nests: Alaska 157, New York 178, Tennessee 162). Chicks were individually marked and swapped for all treatments on day 3 of the nestling period (all days are referred to by days from hatch of first nestling, day 0). Breeding females were captured on the nest on day 4, weighed and measured and aged as either second year or older by plumage (Robertson et al. 1992). A common assessment of immune function in birds is through stimulation of the cell-mediated immune system via a mitogen (Smits et al. 1999) causing an inflammatory response whereby lymphocytes and macrophages infiltrate the site, causing localized swelling (Stadecker et al. 1977; Goto et al. 1978). In each breeding female, 0.15 mg of phytohaemagglutinin (PHA-P) in 30  $\mu$ l of phosphate buffered saline was injected into the patagial wing web of breeding females (Smits and Williams 1999). The thickness of the wing web was measured prior to each injection and then remeasured approximately 48 h post-injection. For both pre and post-injection measurements wing web thickness was measured three times and the average measurement used in all further calculations (repeatability: pre-exposure  $F_{155,156}=87.54$   $P=0.0001$ ,  $R=0.97$ , post-exposure  $F_{155,156}=66.76$   $P=0.0001$ ,  $R=0.91$ ) (Lessells and Boag 1987). While many studies assess PHA response after 24 h (Smits and Williams 1999), pilot data suggested no difference in swelling response between 24 and 48 h ( $t_{1,42} = 1.01$ ,  $P = 0.31$ , unpublished data). We decided to measure PHA response after 48 h in order to minimize disturbance. Cell-mediated immune response was quantified as the difference between post-injection thickness and pre-injection thickness (Smits and Williams 1999). In addition to mounting cell-mediated immune responses to PHA, breeding females were exposed to a humoral immune challenge of sheep red blood cells (Ardia 2005b).

Nestling immune function was assessed on day 10 of the nestling period via an injection of 0.1 mg of phytohaemagglutinin (PHA-P) in 20  $\mu$ l of phosphate buffered saline into the

patagial wing web. The subsequent response to PHA was measured on day 12 in the same manner as for breeding females described above. Repeatabilities for nestling wing web thickness were high: (repeatability: pre-exposure  $F_{824,825}=227.01$   $P=0.0001$ ,  $R=0.95$ , post-exposure  $F_{824,825}=166.88$   $P=0.0001$ ,  $R=0.89$ ).

### Statistical analyses

To compare the relative contributions of maternal and environmental conditions, the effect of common origin and common rearing environment was analyzed using a mixed-model nested ANOVA for each site with random and fixed effects (Littell et al. 1996) following the analyses used by Merilä (1996). Analyses were conducted separately for each site. Each trio of nests that exchanged nestlings was considered a triad (random effect) to control for seasonal changes. In the full model, the main random effects were triad and nest of origin (nested within triad) and the main fixed effect was brood manipulation treatment (reduced, control, enlarged). In addition, the following interaction terms were included: the interaction between brood manipulation treatment\*nest of origin and brood manipulation treatment\*triad. Year was not included in models, because in addition to being redundant with breeding site, the geographically-separated sites used in this experiment show no correlation in environmental conditions during a calendar year when temperature is compared after standardizing lay dates (mean daily daytime temperature: NY vs. TN,  $R=0.04$ ,  $P=0.65$ , NY vs. AK,  $R=0.06$ ,  $P=0.51$ , TN vs. AK,  $R=0.02$ ,  $P=0.77$ ).

To calculate heritability, variance components associated with each random effect in mixed model analyses were estimated using the SAS VARCOMP procedure (SAS 1988) using restricted maximum likelihood estimates. This method of estimating variance components follows Merilä (1996). Within each triad, the variation due to nest of origin estimates  $1/2 V_A$  (additive genetic variance) as well as a quarter of the dominance variance ( $V_D$ ) and pre-experiment maternal effects. To estimate  $V_A$  the variance due to nest of origin was doubled, thus  $V_A$  is an estimate because it also includes a portion of  $V_D$  (Merilä 1996; Christe et al. 2000). The interaction between brood manipulation treatment and nest of origin would indicate that the effect of the brood size manipulation differed among families and was considered the genotype–environment interaction ( $V_{GE}$ ) (Merilä 1996). The error variance estimated other environmental factors, as well as the remainder of  $V_A$  ( $1/2$ ) and  $V_D$  ( $3/4$ ). Environmental variance ( $V_E$ ) consisted of variance caused by the brood size manipulation experiment, triad, the interaction between brood manipulation and triad, as well as the error variance (i.e.  $V_E = \text{experiment} + \text{triad} + \text{experiment*triad} + \text{error}$ ). All these variance components sum to total phenotype variance ( $V_P=V_A+V_E+V_{GE}$ ). Heritability was considered  $h^2=V_A/V_P$ . The coefficient of variation for phenotypic variation ( $V_P$ ) and additive genetic variation ( $V_A$ ) was calculated as  $CV=\sqrt{V/X}$  where  $X$  was the mean immune response to PHA (Houle 1992). Mother–offspring regressions were conducted for each site using linear regression using the mean brood value of related offspring. An empirical bootstrap distribution was generated by resampling the dataset for each site 100,000 times with replacement using H2BOOT (Phillips 2005). Significant differences among heritability estimates for each site were tested with an one-factor (site) ANOVA using the bootstrap dataset. Means  $\pm$  standard deviation are reported.

**Results**

Variance components of nestling immune response

Nestling immune response to PHA did not vary among sites (mean change in wing web thickness in mm±SD; Alaska 1.54±0.21, New York 1.51±0.22, Tennessee 1.56±0.19;  $F_{2, 557}=1.45, P=0.23$ ). However, the relative importance of common origin on nestling immune function varied among sites. In both Alaska and New York, no variance in nestling immune response was explained by common origin, while in Tennessee, common origin explained the plurality of variance (Table 1). Triad, a reflection of seasonal variation within each site, was a predictor of immune response in New York; triad also played a role through its interaction with brood manipulation treatment in Alaska (Table 1). Brood manipulation treatment alone explained little variation in any site (Table 1).

Estimated heritability

Estimated variance components led to a heritability of immune response to PHA in nestlings in Tennessee of  $h^2=0.42$  (95% confidence interval 0.27–0.51), while in New York and Alaska, there was no additive genetic variance; heritability was estimated at 0.00 (0–0.11) for New York and 0.00 (0–0.13) for Alaska (Table 2). Coefficients of phenotypic and additive genetic variation are reported in Table 2. Heritability differed among sites ( $F=17.02, P<0.001$ ). Based on our sample size, we had a power of 0.71 ( $\alpha=0.05$ ) to detect differences in heritability among sites (Klein 1974).

The correlation between the PHA response of breeding females and the response of their offspring differed among sites (Fig. 1). In Tennessee, adult females who produced strong responses tended to have offspring that also produced strong responses ( $F_{1,39}=9.9, P=0.001, \beta=0.11, R^2=0.13$ ), while there was no significant relationship between adult female responses and offspring responses in New York and Alaska (New York  $F_{1,55}=0.22, P=0.70$ ; Alaska  $F_{1,27}=0.20, P=0.79$ ).

**Table 1** Variance components of nestling immune response at three locations across the breeding range

Source	Site											
	Alaska			New York			Tennessee			All Three Sites		
	Df	Var.	%	Df	Var.	%	Df	Var.	%	Df	Var.	%
Triad	38	0.0019	4.8	43	0.0084*	16.7	33	0.0078	10.5	118	0.0028**	7.4
Experiment	2	0.0021	5.3	2	0.0052	10.4	2	0.0036	4.9	2	0.0018	4.8
Origin (triad)	38	0.0000	0.0	43	0.0000	0.0	33	0.0156***	21.1	118	0.0027	7.1
Experiment × Triad	18	0.0064**	15.1	38	0.0051	10.1	16	0.0071	9.6	83	0.0031**	8.2
Experiment × Origin (Triad)	18	0.0030	7.5	38	0.0046	9.1	16	0.0054	7.3	83	0.0017	4.5
Error	78	0.0267	67.3	179	0.0270	53.6	78	0.0345	46.6	354	0.0256	67.6
Site									2		0.0002	0.5
$V_P$		0.0397	100		0.0503	100		0.0740	100		0.0361	100
Model	58	$F = 4.49^{**}$		82	$F = 1.89^{***}$		62	$F = 1.48^*$		205	$F = 1.71^{***}$	
$r^2$		0.48			0.48			0.42			0.50	

**Table 2** Phenotypic ( $V_P$ ), environmental ( $V_E$ ) and additive genetic ( $V_A$ ) components of variation and heritability of immune response to PHA in nestling tree swallows at three sites across their breeding range

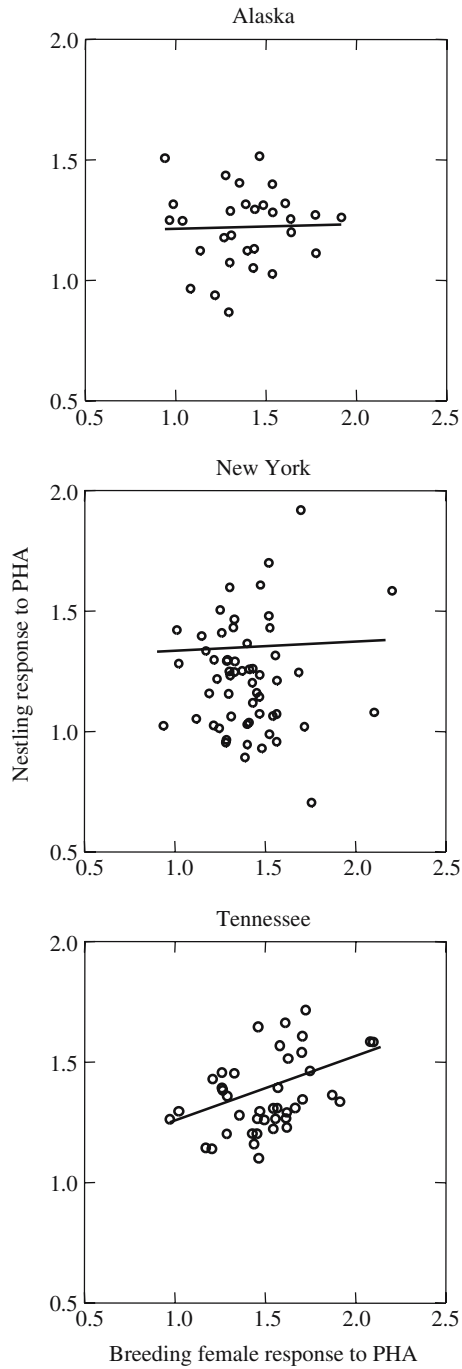
Site	$V_P$	$V_E$	$V_A$	$V_{GE}$	$h^2$	$CV_P$	$CV_A$
Alaska	0.040	0.036	0.000	0.003	0.000	12.9%	0.0%
New York	0.050	0.046	0.000	0.005	0.000	14.8%	0.0%
Tennessee	0.074	0.043	0.031	0.005	0.422	19.9%	11.3%
Sites combined	0.040	0.033	0.005	0.002	0.125	13.8%	4.8%

## Discussion

This study examined variation in the heritability of an important physiological measure, immune function, in the tree swallow. We found that immune function had a heritable component in Tennessee, but not in Alaska or New York, suggesting that the ability of immune function to respond to natural selection may differ among breeding sites of the tree swallow or at least across years. This was reflected in differences in the relative contribution of common origin, with two sites, Alaska and New York, showing no additive genetic variance in nestling immune response. In Tennessee, immune response was strongly dependent on common origin and showed an estimated heritability of  $h^2=0.31$ . This variation in heritability in immune function among sites was supported by mother–offspring regressions, which showed a strong positive relationship in Tennessee and no relationship in New York and Alaska. These mother–offspring regressions are not in themselves direct evidence, due to the measurements being conducted at different life history stages and with different volumes of PHA (though similar ratios of PHA to body size), but they support the overall trend revealed in common origin analyses.

The results are particularly interesting because we found no difference in mean PHA values between sites, even though additive genetic variance varied among sites. Low heritability in Alaska and New York arose because additive genetic variance was zero (Falconer and Mackay 1996). This zero genetic variance in Alaska and New York may suggest a limited evolutionary scope for immune function due to low heritability. Strong selection is believed to reduce additive genetic variation (Fisher 1930), although others have found that traits important to fitness have low heritability due to greater environmental variance, *not* low additive variance (Merilä and Sheldon 2000). In addition, other studies have reported increased genetic variation under stressful environments (Hoffmann and Parsons 1991; Hoffmann and Merilä 1999; Bublly and Loeschke 2002), indicating that conclusions about the relative fitness value of a trait from low levels of heritability are speculative and merit additional study.

It isn't clear whether the differences reported here represent true population differences or yearly differences in heritability. Regardless, they suggest that under some conditions immune function may not be heritable, and thus not subject to natural selection. Examination of why Tennessee might show differences in additive genetic variation suggests that it may be due to differences in resource availability or selection pressure from parasites. Our sites differed in resource availability, with Tennessee showing the lowest food abundance (Ardia 2005b). Differences in parasite exposure among sites may drive differential immune function, either through phenotypic expression or differential genetic influences (Hamilton and Zuk 1982; Møller et al. 1998; Møller et al. 1999). However, without further work, we cannot conclude that the differences reported here represent true differences among sites.



**Fig. 1** Plots of the relationship between the immune response of breeding female tree swallows and the immune response of their natal offspring at three sites across their breeding range

In addition, we found no difference in the effect of the brood manipulation among sites (or across years). The brood manipulation was intended to create greater environmental variation among offspring in access to resources in order to test whether the effect of common origin varied among brood size within each site. The common origin-by-environment interaction reflected how nestlings responded to the brood manipulation within each site by examining whether similar individuals respond differently in different situations. This interaction was not significant in any of the sites, rather variation within each site appeared to be more important.

A drawback to this experiment is that maternal effects, as well as genetics, can underlie the role of common origin. Nestlings were switched among nests at three days of age, so conditions experienced during incubation and early hatching, as well as differences in egg quality, may influence differences among sites. Other work done at these locations revealed differences in the onset of incubation between sites, with females in Tennessee being more likely to begin incubation prior to clutch completion with accompanying shorter incubation periods and greater hatching asynchrony (Ardia et al. 2006). The length of the incubation period can have strong influences on developmental trajectories which in turn can influence immune function (Ricklefs 1992). Another strong maternal influence could be the transfer of immunoglobulins in eggs, which may be higher in cases of high parasitism (Saino et al. 2001) stimulating induced defenses via antibody transfer in eggs (Heeb et al. 1998; Gasparini et al. 2001). In addition, high levels of promiscuity in tree swallows make many nestmates half-siblings (Whittingham et al. 1993; Kempenaers et al. 1998) rather than full siblings, but the lack of any relationship between maternal and offspring immune response at the Alaska and New York sites make this caveat less important. A future approach to this question should consider more strongly controlling for maternal effects (Soler et al. 2003).

This study illustrates that heritability and the role of common origin can vary in the tree swallow. The variation reported here may represent differences among sites or differences among years or both. The lack of heritability of immune response was due to no additive genetic variation in two of the three populations examined. The hypothesis that these differences are due to variation in environmental conditions warrants further testing. In conclusion, studies examining heritability in a single year or a single locale should be cognizant that levels of additive genetic variance may vary over time or space.

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## References

- Ardia DR (2005a) Individual quality mediates tradeoffs between reproductive effort and immune function in tree swallows. *J Anim Ecol* 74:517–524
- Ardia DR (2005b) Tree swallows tradeoff immune function and reproductive effort differently across their range. *Ecology* 86:2040–2046

- Ardia, DR, Cooper, CB, and Dhondt, AA (2006) Warm temperatures lead to early onset of incubation, shorter incubation periods and greater hatching asynchrony in tree swallows at the extremes of their range. *J Avian Biol*, 37:137–142
- Ardia DR, Schat KA, Winkler DW (2003) Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*). *Proc Roy Soc London B* 270:1679–1683
- Brinkhof M, Heeb P, Kölliker M, Richner H (1999) Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc Roy Soc London B* 266:2315–2322
- Bubliy O, Loeschcke V (2002) Effect of low stressful temperature on genetic variation of five quantitative traits in *Drosophila melanogaster*. *Heredity* 89:70–75
- Charmantier A, Garant D (2005) Environmental quality and evolutionary potential: lessons from wild populations. *Proc Roy Soc B-Biol Sci* 272:1415–1425
- Cheng S, Rothschild M, Lamont SJ (1991) Estimates of quantitative genetic parameters of immunological traits in the chicken. *Poultry Sci* 70:2023–2027
- Christe P, de Lope F, Gonzalez G, Saino N, Møller AP (2001) The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* 126:333–338
- Christe P, Møller AP, 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* 85:75–83
- Davison TF (2003) The immunologists' debt to the chicken. *British Poultry Sci* 44:6–21
- de Neve L, Soler J, Pérez-Contreras T, Soler M (2004) Genetic, environmental and maternal effects on magpie nestling-fitness traits under different nutritional conditions: a new experimental approach. *Evol Ecol Res* 6:415–431
- Falconer D, Mackay T (1996) Introduction to quantitative genetics. Longman, London
- Fisher R (1930) The genetical theory of natural selection. Clarendon Press, Oxford
- Gasparini J, McCoy K, Haussy C, Tveraa T, Boulinier T (2001) Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake *Rissa tridactyla*. *Proc Roy Soc London B* 268:647–650
- Goto N, Kadama H, Okada K, Fujimoto Y (1978) Suppression of phytohemagglutinin skin response in thymectomized chickens. *Poultry Sci* 57:246–250
- Hamilton W, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? *Science* 218:384–387
- Heeb P, Werner I, Kölliker M, Richner H (1998) Benefits of induced host responses against an ectoparasite. *Proc Roy Soc London B* 265:51–56
- Hoffmann A, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol Evol* 14:96–101
- Hoffmann A, Parsons P (1991) Evolutionary genetics and environmental stress. Oxford University Press, Oxford
- Hörak P, Lea T, Ots I, Møller AP (1999) Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia* 121:316–322
- Houle D (1992) Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204
- Kempnaers B, Lanctot RB, Robertson RJ (1998) Certainty of paternity and paternal investment in eastern bluebirds and tree swallows *Anim Behav* 55:845–860
- Klein TW (1974) Heritability and genetic correlation: statistical power, population comparisons, and sample size. *Behav Genet* 4: 171–189
- Lessells C, Boag P (1987) Unrepeatable repeatabilities. *Auk* 104:116–121
- Littell R, Milliken G, Stroup W, Wolfinger R (1996) SAS system for mixed models. SAS Institute, Cary, NC
- Martin TE, Møller AP, Merino S, Clobert J (2001) Does clutch size evolve in response to parasites and immunocompetence. *Proc National Acad Sci* 98:2071–2076
- Merilä J (1996) Genetic variation in offspring condition: an experiment. *Functional Ecol* 10:465–474
- Merilä J, Sheldon BC (2000) Lifetime reproductive success and heritability in nature. *Am Nat* 155:301–310
- Møller AP, Christe P, Lux E (1999) Parasitism, host immune function, and sexual selection. *Quarterly Rev Biol* 74:3–20
- Møller AP, Saino N (2004) Immune response and survival. *Oikos* 104:299–304
- Møller AP, Sorci G, Erritzoe J (1998) Sexual dimorphism in immune defense. *Am Nat* 152:605–619
- Mousseau T, Roff D (1987) Natural selection and the heritability of fitness components. *Heredity* 59:181–197
- Nath M, Singh B, Saxena V, Singh R, Roy A (2001) Genetic analysis of concanavalin-A response in broilers. *J Appl Anim Res* 20:171–180
- Phillips P (2005) H2BOOT. University of Oregon, Corvallis, OR
- Raberg L, Stjernman M, (2003) Natural selection on immune responsiveness in blue tits *Parus caeruleus*. *Evolution* 57:1670–1678

- Ricklefs RE, (1992) Embryonic development period and the prevalence of avian blood parasites. *Proc Nat Acad Sci* 89:4722–4725
- Robertson RJ, Stutchbury BJ, Cohen RR (1992) Tree swallow. In: Poole A, Stettenheim P, Gill F (eds) *Birds of North America*, vol 11. Academy of Natural Sciences/Cornell Laboratory of Ornithology, Philadelphia/Ithaca pp 27
- Roff D, Mostoway S, Fairbairn D (2002) The evolution of trade-offs: testing prediction on response to selection and environmental variation. *Evolution* 56:84–95
- Saino N, Calza S, Møller AP (1997) Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J Anim Ecol* 66:827–836
- Saino N, Martinelli R, Møller AP (2001) Immunoglobulin plasma concentration in relation to egg laying and mate ornamentation of female barn swallows (*Hirunda rustica*). *J Evol Biol* 14:95–109
- SAS (1988) *SAS/STAT user's guide*. SAS Institute, Cary, NC
- Smits J, Bortolotti G, Tella J (1999) Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct Ecol* 13:567–572
- Smits J, Williams TD (1999) Validation of immunotoxicology techniques in passerine chicks exposed to oil sands tailings water. *Ecotoxicol Environ Safe* 44:105–112
- Soler J, Moreno J, Pacheco ND (2003) Environmental, genetic and maternal components of immunocompetence of nestling pied flycatchers from a cross-fostering study. *Evol Ecol Res* 5:259–272
- Stadecker M, Lukic M, Dvorak A, Leaskowitz S (1977) The cutaneous basophil response to phytohaemagglutinin in chickens. *J Immunol* 118:1564–1568
- Taylor R, Cotter P, Wing R, Briles W (1987) Major histocompatibility B complex and sex effects on the phytohaemagglutinin wattle response. *Anim Genet* 18:343–350
- Tella J, Bortolotti G, Forero M, Dawson R (2000) Environmental and genetic variation in T-cell-mediated immune response of fledgling American kestrels. *Oecologia* 123:453–459
- Tella J, Scheuerlein A, Ricklefs RE (2002) Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proc Roy Soc London B* 269:1059–1066
- Warner C, Meeker D, Rothschild M (1987) Genetic control of immune responsiveness: a review of its use as a tool for selection for disease resistance. *J Anim Sci* 64:394–406
- Whittingham LA, Dunn PO, Robertson RJ (1993) Confidence of paternity and male paternal care: an experimental study in tree swallows. *Anim Behav* 46:139–147
- Zekarias B, Ter Huurne A, Landman W, Rebel J, Pol J, Gruys E (2002) Immunological basis of differences in disease resistance in the chicken. *Vet Res* 33:109–125