

Daniel R. Ardía

Cross-fostering reveals an effect of spleen size and nest temperatures on immune responses in nestling European starlings

Received: 3 November 2004 / Accepted: 29 March 2005 / Published online: 11 May 2005
© Springer-Verlag 2005

Abstract Immunocompetence may be a good measure of offspring quality, however, factors affecting variation in immune responses are not clear. Research suggests that immune function can vary due to differences in genetics, development conditions and individual quality. Here, I examined factors affecting variation in immune response among nestling European starlings through a split-nest cross-fostering brood manipulation that included two important covariates: spleen size and nest temperatures. Immunocompetence was assessed via a cell-mediated immune response to phytohaemagglutinin (PHA). This paper provides the first direct evidence that individuals with large spleens also mount strong immune responses. Exposure to PHA did not cause splenomegaly, as there was no difference in spleen size between control birds and those injected with PHA. Offspring immune function was affected by common origin and by rearing environment, though rearing environment appeared to exert its influence only through nest temperatures. A comparison of the immune performance of siblings reared in their home nest versus those reared in other nests revealed a strong effect of maternal quality. As the difference in natal clutch size increased, the magnitude of the difference in immune performance between home-reared nestlings versus out-reared nestlings increased. Overall, nestling immune function appears to be determined by the combination of genetic, maternal and environmental effects.

Keywords Cell-mediated immune function · Cross-fostering · Offspring quality · Rearing environment · *Sturnus vulgaris*

Introduction

Parents make multiple contributions to their offspring, each of which affects the probability that offspring will survive to reproduce. For example, many studies in birds have shown that body condition (an index of body mass corrected for structural size) is a predictor of fledging survival (Perrins 1964; Dhondt 1971; Hochachka and Smith 1991; Linden et al. 1992, Adriaensen et al. 1998). The strength and responsiveness of the immune system has provided an additional currency to assess offspring quality, as levels of offspring immune function have been linked to return rates or yearly survival (Hörak et al. 1999; Christe et al. 1998). The ability to mount a strong immune response should have a strong genetic component, given the role of the major histocompatibility complex (MHC) genes in the functioning of the cellular immune response (Taylor et al. 1987; Warner et al. 1987; Cheng and Lamont 1988). However, experimental work has shown that rearing environment often explains more variation among nestlings than does nest of origin, primarily through the indirect influence of nestling body mass (Tella et al. 2000; Brinkhof et al. 1999). Body mass may influence immune response by affecting nutritional condition (Lochmiller et al. 1993; Saino et al. 1997; Christe et al. 1998; Hoi-Leitner et al. 2001). Therefore, to assess offspring quality as the interaction between condition and immune responses, it is important to consider factors influencing both measures.

Here, I expand on previous studies through a split-nest cross-fostering brood manipulation experiment in European starlings (*Sturnus vulgaris*). To better assess the relationship between condition and immune function, I examine the role of common origin and common rearing environment by including two additional

Communicated by Carol Vleck

D. R. Ardía (✉)
Department of Ecology and Evolutionary Biology,
Cornell University, Ithaca, NY 14853, USA

Present address: D. R. Ardía
Program in Organismic and Evolutionary Biology,
Morrill Science Center, University of Massachusetts,
319 Morrill Science Building, Amherst, MA 01003, USA
E-mail: ardia@bio.umass.edu
Tel.: +1-413-5450035

variables: spleen size and temperature conditions during development. Including these as covariates can help explain how common origin and common rearing environment influence immune responses by increasing the amount of variation explained among individuals and providing evidence of exactly what factors vary among the nests that contribute to the differences.

The spleen, through its role in filtering blood and storing and producing lymphocytes, is an important organ in the avian immune system (John 1994), and, accordingly, many have suggested that spleens should be larger in individuals with strong immune function (Møller et al. 1998a, b; Møller and Erritøe 2000; Blanco et al. 2001; Blount et al. 2003). However, Smith and Hunt (2004) expressed concern that broad conclusions regarding spleen size were based on the untested assumption that larger spleens indicate stronger immune function. Exposure to parasites might induce spleen growth (splenomegaly), and thus differences in spleen size could be due to differing histories of parasite exposure, but not due to underlying differences in baseline immunocompetence among individuals (John 1994, 1995; Smith and Hunt 2004).

Therefore, to determine whether direct comparisons between spleen size and immune function are warranted, I first tested the hypothesis that exposure to an immune challenge leads to increased spleen size. I then tested (1) whether individuals with large spleens indeed mount stronger immune responses and how spleen size would be influenced by body condition, (2) how the influence of common origin and common rearing environment might vary when including spleen size and nest temperatures as covariates and (3) how the relationship between spleen size and immune function varied along an experimental gradient of ectoparasite exposure, as ectoparasites can affect nestling immune response through stimulating the immune system or causing immunosuppression (Christe et al. 1998, Blanco et al. 2001).

The second important covariate included in this study was nest temperatures, as temperature conditions during offspring development can have both direct and indirect effects on immune function. For example, heat stress reduces both cell-mediated immunity and macrophage activity in chickens (Dietert et al. 1994, Miller and Qureshi 1992). Temperatures outside the thermoneutral zone can depress immune function (Henken et al. 1983, Donker et al. 1990) as well as have significant sublethal effects on energy costs and body condition (Visser 1998), particularly during ontogeny of thermoregulation (Kirkley and Gessaman 1990), and these stresses may have indirect effects on immune function (Apanius 1998).

It is also important to consider variation among individuals in their quality, as well as their conditions of development. In European starlings, large clutch size and early clutch initiation dates predict high reproductive success (Christians et al. 2001; Smith 2004). To examine in more detail the role of parental quality, I compared the immune performance of nestlings reared

in their home nest versus their siblings (likely half-siblings or full siblings as parasitic eggs were removed) reared in other nest as a function of differences between parents in their quality. I predicted that as the magnitude of the difference in natal clutch size differs among females, the difference in immune performance among cross-fostered siblings would also increase.

Materials and methods

Study area and study species

The research took place on a 2500-acre sheep and cattle farm in the Belmont Hills, Lower Hutt, New Zealand (41° 10'S, 88° 53'W) from October 2000 to December 2000. The European starling (*Sturnus vulgaris* Linnaeus) is a cavity-nesting passerine native to Europe and Central Asia. Starlings, which were introduced to New Zealand in early twentieth century (Flux and Flux 1981), breed in Belmont in reconfigured ventilation shafts in concrete munitions bunkers built by the US Army during World War II. Extensive research on starlings has been conducted at the site (Flux and Flux 1981, Thompson and Flux 1988, Thompson et al. 1993).

Experimental procedure

I checked the nests daily to determine the date of clutch initiation and clutch size, and I returned to the nests at the end of incubation to determine hatching date. To manipulate parental effort, I randomly assigned nests with the same hatching date to one of three treatments: (1) enlarged ($N=11$), (2) reduced ($N=11$), or (3) control ($N=12$) to create broods that were roughly 40% larger or smaller than original clutch size (e.g. on average, 3, 5 and 7 nestlings on day 4). Inclement weather reduced brood sizes between day 4 and day 20 to produce lower number of chicks on day 20 (enlarged = 48 chicks, reduced = 21 chicks, control = 35 chicks); there were no differences among treatments in the probability of brood reduction. Chicks were individually marked with small dabs of nail polish on their claws and swapped for all treatments on day 3 of the nestling period (all days are referred to by days from the hatch of first nestling). Within each nest, chicks were randomly selected in order to minimize bias due to hatching-order differences among chicks. Each nest contained a mix of nestlings that hatched in the nest and the nestlings that hatched in other nests. When more than one egg was found in a nest over a 24-h cycle ($N=25$), one egg was always markedly different in color and shape and was then removed, providing a reliable method of ensuring that only eggs laid by the attending females remained in the nest (Brown and Sherman 1989, J.E.C. Flux, personal communication).

In addition, each nest was assigned to (1) ectoparasite reduction ($N=16$) or (2) sham control ($N=17$). In the ectoparasite reduction treatment, I placed three 5 g pieces of foam impregnated with 18.6% dichlorvos and 2,2-dichlorovinyl dimethyl phosphate (Prozap Insect Guard, Loveland, CO, USA) into the nesting material between 5 cm and 10 cm from the nest cup. Control nests were treated with three 5 g pieces of inert foam. Nests were allocated randomly to treatments; there were no differences in natal clutch size or clutch initiation date among brood manipulation treatments nor between ectoparasite treatments ($P_s > 0.20$); in addition, nests used in this experiment did not differ in clutch size or lay date from the population as a whole.

On day 19, I measured the cell-mediated immune response of each nestling with an immunochallenge of 0.15 mg of phytohaemagglutinin (PHA) suspended in 30 μ l of phosphate-buffered saline injected in the right wing web (Smits and Williams 1999). PHA is a mitogen which stimulates the proliferation of resting T-lymphocytes and response to PHA is a standard measure of immunocompetence (Smits and Williams 1999). The response is the ratio of the thickness of the wing web 24 h (± 1 h) after injection divided by the thickness before injection. I measured the thickness of the wing web to the nearest 0.01 mm using digital calipers. At each occasion, I took three measurements and used the average of the three measurements (Repeatabilities: pre-exposure $R_i = 0.80$, $F_{102,103} = 1.50$, $P < 0.01$, post-exposure $R_i = 0.76$, $F_{102,103} = 1.63$, $P = 0.02$). To examine the effects of PHA on spleen size, 12 nestlings (in five different nests) were given an injection of 30 μ l of phosphate-buffered saline in the right wing web and then monitored and handled in the same manner as experimental nestlings.

On day 20, I measured nestling body mass to the nearest 0.1 g with an electronic balance and the length of the tarsus using digital calipers to the nearest 0.01 mm. Nestling residual mass was calculated as the residual of a regression of body mass versus tarsus length ($F_{1,101} = 9.97$, $P = 0.001$, $R^2 = 0.54$, $N = 104$).

I visited each nest on days 4, 8, 12, 16, 19 and 20 to measure nest temperatures and examine each nestling for ectoparasites (e.g. feather lice, blowfly larvae) through examination of wing and body feathers. Nestlings with ectoparasites were initially scored on a scale of 1–5, but because ectoparasite load was low, these scores were converted to a score or “Yes” or “No” for feather lice and blowfly larvae respectively. Nestlings were also checked for the presence of mites and ticks and the presence of *Carnus hemapterus* (Liker et al. 2001); none were present. Nest temperatures were measured to the nearest 0.1°C with a thermocouple thermometer. Air temperature was measured in nests at the base of the nest cup while occupied by nestlings and then after nestlings had been removed and air temperature had cooled to the ambient temperature of the microclimate of the nest.

On day 20, following measurements, I collected nestlings via overetherization and weighed the body and the spleen to the nearest 0.1 g with an electronic balance. In addition, spleen length and width were measured to the nearest 0.01 mm with digital calipers, and spleen volume was calculated based on the shape of the spleen as an ellipsoid (Brown and Brown 2002). Residual spleen volume was calculated from a regression of body mass versus spleen volume ($F_{1,101} = 43.39$, $P < 0.0001$, $R^2 = 0.49$, $N = 104$).

Statistical analyses

Factors affecting nestling immune response to PHA were analyzed using a mixed model (SAS PROC MIXED, Littell et al. 1996), with nest of origin and nest of rearing as random factors and the following fixed effects: brood manipulation treatment (reduced, control, enlarged), ectoparasite treatment (yes, no), clutch initiation date, body mass, tarsus length, spleen volume, ectoparasite load (yes, no), occupied nest temperature and the temperature difference between occupied and empty nest temperatures. Interactions between variables were included in the model and removed sequentially by highest significance value. Removing nonsignificant interactions did not change significance of main effects.

The effect of common origin on nestling immune response was analyzed through the random effects of nest of origin and nest of rearing from mixed model analyses. Random effects were tested by subtracting the -2 Log Likelihood scores of a model containing all random effects from the -2 Log Likelihood score of the model minus the effect being tested (Littell et al. 1996). The difference in the -2 Log Likelihood scores between the full model and the model minus the variable of concern is computed as a Chi-square value $df = 1$. Degrees of freedom are calculated by the model for each variable separately, these are reported with Table 1 (Littell et al. 1996). To compare the relative contribution of each random effect to explaining variance not explained by fixed effects, I report covariate parameter estimates. To test whether including nest temperatures in the overall model influenced the random effects of common origin and common environment on PHA response, I retested random effects without nest temperatures. Nest temperatures were initially grouped into three periods of the nesting cycle (early days 4–8, mid days 12–16 and late days 19–20), but there was no difference in conclusions when nest temperatures were analyzed in three groupings or as a single average temperature, so average temperature over the entire nestling period was used to preserve degrees of freedom in analyses.

Because significance tests based on residuals can be biased (Darlington and Smulders 2001), I conducted all significance tests using raw body mass, tarsus length and spleen volume. However, in order to illustrate relationships among variables correcting body mass and spleen volume for body size, I report residual body mass and residual spleen volume in figures.

Table 1 Fixed effects from mixed model analysis of factors affecting European starling nestling immune response to PHA. Random effects of natal nest and nest of rearing were both significant ($P_s < 0.05$)

Fixed effect	Standardized parameter estimate	Standard error	Num <i>df</i>	Den <i>df</i>	<i>F</i>	<i>P</i>
Brood size manipulation			2	29	0.14	0.86
Ectoparasite manipulation			1	28	0.04	0.84
Natal clutch initiation date	-0.03133	0.02247	1	27	1.95	0.17
Natal clutch size	0.01626	0.04900	1	30	0.11	0.74
Body mass	0.003873	0.00454	1	83	1.26	0.21
Tarsus length	0.02442	0.02143	1	70	0.76	0.45
Spleen volume	0.000571	0.00335	1	64	7.23	0.001
Presence of feather lice	0.006572	0.00017	1	26	0.19	0.69
Presence of blowfly larvae	0.008205	0.00023	1	26	0.17	0.73
Occupied nest temperature	0.02562	0.01411	1	27	0.86	0.54
Temperature difference between occupied and empty nest	0.005682	0.007134	1	27	4.45	0.04
Brood size day 12	0.01558	0.05423	1	27	0.34	0.63
Intercept	2.412	0.8297				

I used multiple regression to test what factors affected the difference between nestling immune response in home-reared offspring (those hatched and reared in the same box) versus out-reared nestlings (those hatched in the same box as home-reared nestlings but reared in a different box). The model also contained the following fixed effects: clutch initiation date, natal clutch size of the home nest, natal clutch size of the out-reared nest, the difference in natal clutch sizes between nests, the difference between the home-reared nestlings and out-reared nestlings in body mass, spleen volume, and nest temperatures during the early, middle and late nestling stages in both home and out-reared nests. To avoid pseudoreplication, for each nest, I used the average values for all the nestlings in each category in each nest. Because brood reduction sometimes led to mortality of siblings, I only used nests where at least one sibling remained in both a home nest and an out-reared nest ($N=19$).

Results

Does exposure to PHA induce splenomegaly?

Increased spleen size was not due to exposure to PHA, as there was no difference in spleen volume between individuals exposed to PHA and individuals that were not (mean spleen volume (mm^3) \pm SD: PHA 206.7 ± 18.4 , $N=35$; not exposed to PHA 203.4 ± 13.3 , $N=12$; spleen volume $F_{1,44}=0.22$, $P=0.64$; effect of body mass $F_{1,44}=4.65$, $P=0.03$; body mass difference between injected and noninjected chicks $F_{1,45}=0.18$, $P=0.67$). The lack of significant difference in spleen volume between individuals exposed to PHA and not exposed to PHA was not due to a lack of power; there was a power of 0.78 to detect differences between groups based on the sample sizes and observed means and variation.

Factors predicting nestling immune response

There was no direct effect of brood manipulation treatment on nestling immune response to PHA (Table 1). There was also no effect of placing insecticide-impregnated foam into nests on either ectoparasite load observed on nestlings or on chick parameters (Table 1). The exposure of nestlings to the impregnated foam had no effect on nestling body mass, residual body mass, PHA or spleen volume ($F_s < 1.0$, $P_s > 0.45$). Given the sample size and variation observed, there was sufficient power (at 0.95) to detect a difference in all variables assessed (PHA: calculated power difference between = 0.45, actual difference = 0.21; body mass calculated = 5.93, actual = 0.8; spleen volume calculated = 53.2 actual = 5.6; residual body mass calculated = 5.39 actual = 0.32).

Rather, cell-mediated immune response to PHA was related to spleen volume and temperature conditions in the nest (Table 1). Nestlings with greater spleen volume mounted stronger PHA responses (Table 1, Fig. 1a). There was no effect of either ectoparasite treatment or ectoparasite load on immune response (Table 1), though most individuals had no infestation of ectoparasites (feather lice: 93 of 104 individuals; blowflies 98 of 104). There was no direct effect of either absolute body mass or tarsus length on PHA response (Table 1); however, there was a significant relationship between residual body mass and PHA response (GLM $F_{1,101}=3.63$, $P=0.01$, $R^2=0.04$, slope = 6.22; Fig. 1b).

Nest of origin had a significant influence on PHA response in the mixed model analysis; removal of natal nest resulted in a significant change in the -2 Log Likelihood values ($\chi^2_1=9.01$, $P < 0.01$, 40% of variance explained by natal nest), but nest of rearing did not ($\chi^2_1=0.8$, $P > 0.05$, 6.5% of variance explained). However, removal of the occupied nest temperature from the model caused the random effect of both natal nest and nest of rearing to be significant (natal nest $\chi^2_1=8.6$,

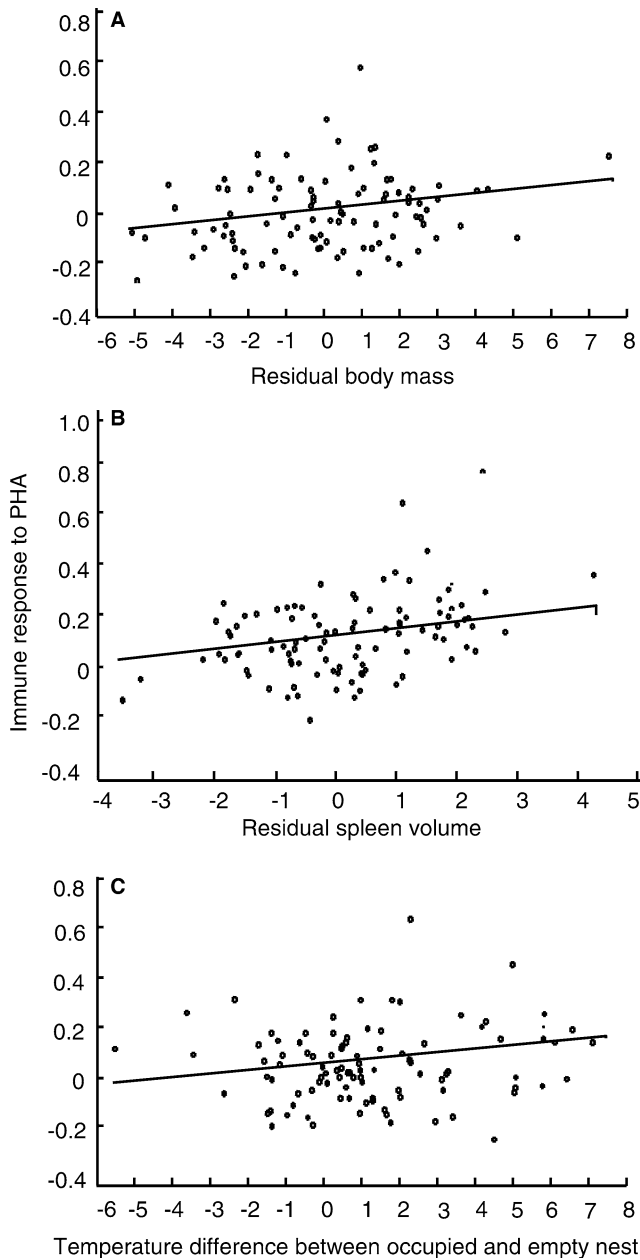


Fig. 1 Effect of nestling measures on cell-mediated immune response to PHA in European starling nestlings. Data presented partial regression plots. Partial regression plots for each variable was created by regressing the first variable of interest against the other independent variables minus nestling immune response and by plotting the residual of this regression against the residuals of a regression of the first variable of interest against all other variables including nestling immune response. **a** Residual body mass ($F_{1,101} = 3.63$, $P = 0.01$, $R^2 = 0.04$, slope = 6.22). **b** Residual spleen volume ($F_{1,64} = 7.23$, $P = 0.001$, $R^2 = 0.06$, slope = 0.000571). **c** Residual temperature difference between occupied and empty nests ($F_{1,27} = 4.45$, $P = 0.04$, $R^2 = 0.03$, slope = 0.005682)

$P < 0.01$; nest of rearing $\chi^2_1 = 10.3$, $P < 0.01$), suggesting that temperature can mediate the effect of rearing conditions on nestling immune function. Spleen volume was significantly affected by box of origin but not by box of rearing (spleen volume: natal nest $\chi^2_1 = 4.1$, $P = 0.04$,

20.1% of variance; nest of rearing $\chi^2_1 = 0.9$, $P > 0.05$, 6.7% of variance).

Absolute nest temperatures during any development period had no direct effect on PHA responses, but nestlings reared in nests with a greater differential in temperature between the occupied nest temperature and the empty nest temperature tended to mount higher immune responses than did nestlings in nests with a smaller temperature differential (Fig. 1c), even when correcting for differences in brood size (effect of brood size $F_{1,19} = 1.67$, $P = 0.46$).

Immune performance of related nestlings

A multiple regression analysis of the immune performance between nestlings hatched and reared in their home nest (hereafter referred to as home-reared) versus nestlings hatched in the home nest but reared elsewhere (out-reared) revealed that both common origin and rearing environment play a role (overall model: $F_{5,14} = 6.61$, $P = 0.002$, $R^2 = 0.56$, $N = 19$ nests). The difference in residual body mass between home-reared versus out-reared offspring was the strongest predictor of this difference in immune performance; as the magnitude of the difference in body mass between related nestlings increased so did the difference in immune performance (Fig. 2a; $F_{1,14} = 6.79$, $P = 0.02$, standardized parameter estimate = 0.019, partial- $R^2 = 0.36$). Maternal quality played a role as home-reared nestlings reared in nests with a large positive difference in natal clutch size between home-reared and out-reared nests tended to mount stronger immune responses than did out-reared nestlings (Fig. 2b, $F_{1,14} = 12.33$, $P = 0.003$, standardized parameter estimate = 0.139, partial- $R^2 = 0.17$). In addition, differences in immune performance of home-reared nestlings was the greatest in relation to out-reared nestlings when home nest temperatures during the late nestling stage were the highest, suggesting both a role of parental care as well as absolute conditions (Fig. 2c, $F_{1,14} = 5.76$, $P = 0.03$, standardized parameter estimate = -0.039, partial- $R^2 = 0.08$). There was no consistent variation in spleen size between home-reared and out-reared offspring (overall model $F_{5,14} = 0.33$, $P = 0.88$).

Discussion

Variation among European starling nestlings in cell-mediated immune response to PHA appears to be strongly influenced by physiological condition (reflected in spleen volume and body condition) as well as through maternal influences (reflected in nest temperatures and individual quality). Both common origin and common rearing environment influenced immune responses. Common origin appeared to influence immune function through genetic influences and origin-influenced physiology, namely spleen volume. The effect of common

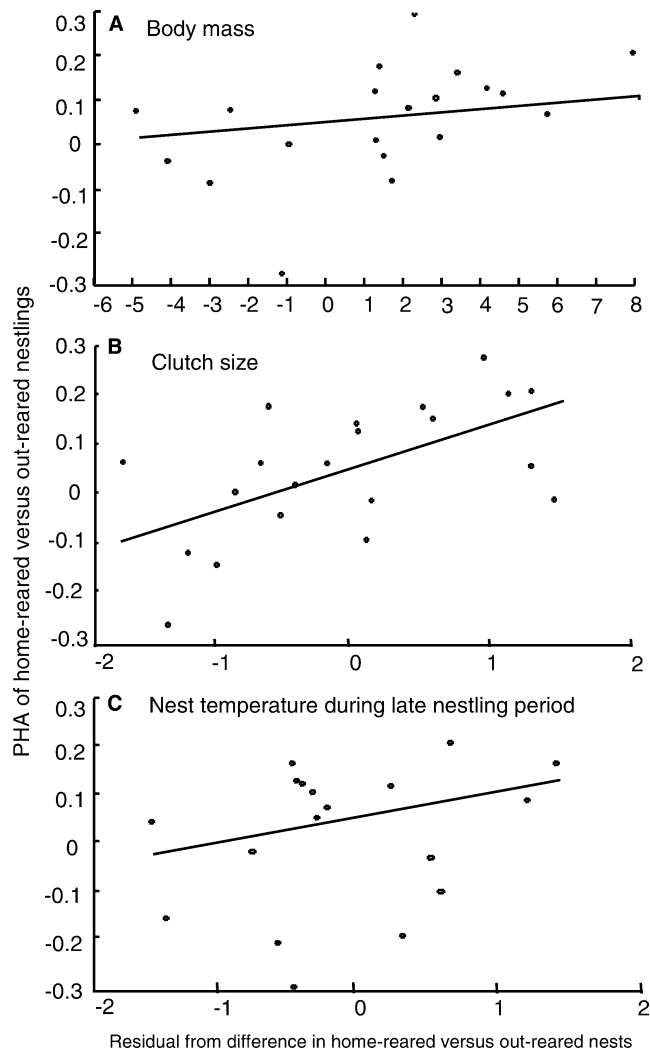


Fig. 2 Effect of nestling measures on difference in cell-mediated immune response to PHA between home-reared and out-reared nestlings. Data presented are from partial regression plots. Partial regression plots for each variable was created by regressing the first variable of interest against the other independent variables minus nestling immune response and by plotting the residual of this regression against the residuals of a regression of the first variable of interest against all other variables including nestling immune response. **a** Residual difference in residual body mass. **b** Residual difference in natal clutch size. **c** Residual difference in occupied nest temperature during late nestling period

rearing environment on nestling immune function was detected through measuring nest temperatures with no significant unexplained rearing environment effects. These results build upon the previous studies by providing better understanding of key variables that influence the role of nature versus nurture in determining an important measure of offspring quality.

The first determinants of an individual nestling's immune function are the inherited components of the immune system such as T-cell reactivity, MHC diversity, and absolute T-cell numbers, all of which are controlled in large measure by genetic influences (Taylor et al. 1987; Warner et al. 1987; Cheng and Lamont 1988). However,

the expression of the immune system could be influenced by the size of immune system organs, such as spleen size. The finding that spleen size was strongly influenced by common origin but not by rearing conditions suggests that the size of the spleen is an additional underlying mechanism helping explain the role of common origin on nestling immune responses.

The next determinant of immune performance is the availability of resources for allocation to immune response. Residual body mass of chicks was linked with immune response and helped explain differences among related nestlings reared in different nests, underscoring the role of resource allocation independent of any genetic influences on body mass. It is unclear, however, if residual body mass directly affects immune function or is itself correlated with an as yet undetermined variable that directly affects immune function. Immune performance has direct energetic costs, and nestlings with high residual body mass would likely have more energy to devote to immune responses.

Directly comparing immune performance among related nestlings reared in different nests allowed for a determination of the role of environmental conditions as influenced by parents. Differences in immune performance between full- or half-siblings should be more attributable to the development conditions, and not to the genetic differences. As the differences in conditions between nests increased, the differences in immune performance among cross-fostered nestlings increased. Female quality exerts an effect through the differences among females in timing of breeding and natal clutch size, but the exact link between female quality and offspring quality is unclear. Certainly, higher quality females can provide more food (reflected in residual body mass) and better development conditions (reflected in temperature conditions). In addition, high-quality individuals may also be bringing higher quality food. Availability of micronutrients, vitamins and protein can provide immunological benefits (Klasing 1998); high-quality females may be better at finding and procuring nutrients such as Linoleic acid, iron, or vitamin A for their offspring. There is evidence from barn swallows that the chicks supplemented with high-protein diets had higher immune function, but not necessarily higher residual body mass (Saino et al. 1997). The role of parental provisioning strategies in wild birds and its influence on immune function remains unknown.

Differences in residual body mass alone do not explain the influence of common rearing environment on immune function. In this study, however, when nest temperatures are not included in the mixed model analyses, the effect of rearing environment on immune function becomes significant, suggesting that the variation in rearing conditions is explained by nest temperatures. This suggests that nestling immune response is an interaction among resource allocation, environmental conditions and maternal quality.

In addition, nest temperatures played an indirect role, as immune performance of siblings was linked to the

degree to which nest temperatures were raised above ambient temperatures—a relative measure that is not indicative of absolute development conditions. The effect of nest temperatures was not due to differences in brood size among nests. Given the energy required by both parents and offspring to maintain nest temperatures against a stronger thermal gradient, it is likely that the association between temperature differences and immune performance is correlated with female quality rather than a direct relationship. As nest temperatures during the nestling cycle did not differ in effect, this supports the role of maternal quality, as high-quality females may have more time available for brooding young nestlings and providing critical food supplies during the periods of inclement weather for older nestlings. Further studies should experimentally manipulate nest temperatures to examine the direct role of temperature conditions.

This is the first study to show that spleen size and immune function are positively linked in the same individual, as individuals with large spleens tended to mount stronger immune responses. Because of its role in storing and producing lymphocytes, a larger spleen may either provide or be correlated with a higher number of resting T-cells, the population of lymphocytes mobilized by exposure to PHA. Interestingly, spleen size was affected only by common origin, suggesting a strong heritable component to spleen size (John 1994, 1995). Thus, spleen size appears to exert an independent effect on immune function, unrelated to the factors (such as temperature and offspring numbers) that influence residual body mass.

It was hoped that spleen size and immune function could be analyzed along a gradient of exposure to ectoparasites, as parasitic infection has been shown to both activate the immune system and stimulate spleen growth (John 1994; Brown and Brown 2002). However, the individuals at my study site had very low exposure to ectoparasites, thus limiting my ability to examine this relationship experimentally. Low ectoparasite load may in fact have been fortuitous as I was able to examine the relationship between spleen size and immune function in an introduced population with low levels of parasite-induced splenomegaly, thus allowing for comparisons of “natural-sized” spleens. Low levels of ectoparasitism may have been a reason why immune challenge did not cause splenomegaly. Further research might repeat this study to examine individual variation in immune response and spleen size at higher ectoparasite levels. Overall, my results suggest that the dynamics of spleen size are complicated and that spleen size as a surrogate for immune function should be used with caution, especially when making comparisons among species.

Spleen volume may be an excellent measure to ask certain questions, such as the consequences of ectoparasite exposure, factors affecting immune response within a single species, or physiological tradeoffs in resource allocation to the immune system. However,

these results suggest that comparative studies of the role of immune function in life history evolution should use an actual measure of immune function rather than spleen volume, except in the opportunistic collection of individuals. In this study, there was more variation in spleen volume than in immune response (coefficient of variation 41.8% for spleen volume vs. 16.1% for PHA response) and this level of variation represents spleen volume in a single year, during a single time of year, and in an introduced species with low ectoparasite exposure. This strongly suggests that studies with small sample sizes comparing spleen volume among species will be greatly limited by individual variation and should be done with considerable caution.

In summary, parents clearly influence nestling immune function through genetic components of the immune system such as reactivity and MHC diversity, as well as spleen size. But this research also shows that the conditions that parents create influence the development and expression of the immune system through offspring reserves and temperature conditions, as well as factors as yet undetermined. Additional work should focus on directly manipulating environmental conditions (particularly nest temperatures) and ectoparasite loads as well as recognizing the critical role of maternal effects to consider individual-level allocation tradeoffs made by both parents and offspring. These factors themselves appear to be under the influence of maternal quality, indicating that future cross-fostering studies should consider differences among parents in their quality when interpreting differences among individuals.

Acknowledgements I am grateful to John and Meg Flux for letting me work at the Belmont field site and for their generosity of time and advice, and Nathaniel Taylor for assistance under difficult field conditions. Elizabeth Atkinson and Fabio provided additional support. This manuscript was improved through discussions with Andre Dhondt, David Winkler, Wesley Hochachka, Karel Schat, Matt Wasson, Dana Hawley, Becca Safran and Mark Hauber. Victor Apanius, Charles Brown and Carol Vleck made comments that substantively improved this manuscript. The U.S. Environmental Protection Agency provided financial support. This work was carried out under an Animal Care Protocol approved by the Cornell Center for Research Animal Resources.

References

- Adriaensen F, Dhondt AA, Van Dongen S, Lens S, Matthysen E (1998) Stabilizing selection on blue tit fledgling mass in the presence of sparrowhawks. *Proc R Soc Lond B Biol Sci* 265:1011–1016
- Apanius V (1998) Ontogeny of immune function. In: Starck JM, Ricklefs RE (eds) *Evolution within the altricial-precocial spectrum*. Oxford University Press, Oxford, pp 203–222
- Blanco G, de la Puente J, Corroto M, Baz A, Cola J (2001) Condition-dependent immune defence in the Magpie: how important is ectoparasitism? *Biol J Linnean Soc* 72:279–286
- Blount JD, Houston DC, Møller AP, Wright J (2003) Do individual branches of immune defence correlate? A comparative case study of scavenging and non-scavenging birds. *Oikos* 102:340–350

- Brinkhof MWG, Heeb P, Kölliker M, Richner H (1999) Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc R Soc Lond B Biol Sci* 266: 2315–2322
- Brown CR, Brown MB (2002) Spleen volume varies with colony size and parasite load in a colonial bird. *Proc R Soc Lond B* 269:1367–1373
- Brown CR, Sherman LC (1989) Variation in the appearance of swallow eggs and the detection of intraspecific brood parasitism. *Condor* 91:620–627
- Cheng S, Lamont SJ (1988) Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poul Sci* 67:989–995
- Christe P, Møller AP, de Lope F (1998) Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos* 83:175–179
- Christians JK, Evanson M, Aiken JJ (2001) Seasonal decline in clutch size in European starlings: A novel randomization test to distinguish between the timing and quality hypotheses. *J Anim Ecol* 70:1080–1087
- Darlington RB, Smulders TV (2001) Problems with residual analysis. *Anim Behav* 62:599–602
- Dhondt AA (1971) The regulation of numbers in Belgian populations of great tits. In: den Boer PJ, Gradwell JR (eds) *Dynamics of populations*. Advanced Study Institute, Wageningen, pp 532–547
- Dietert RR, Golemboski KA, Austic RE (1994) Environment-immune interactions. *Poult Sci* 73:1062–1076
- Donker RA, Nieuwland MGB, Vanderzijpp AJ (1990) Heat-stress influences on antibody-production in chicken lines selected for high and low immune responsiveness. *Poult Sci* 69:599–607
- Flux JEC, Flux MM (1981) Artificial selection and gene flow in wild starlings, *Sturnus vulgaris*. *Naturwissenschaften* 69: 96–97
- Hörak P, Tegelmann L, Ots I, Møller AP (1999) Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia* 121:316–322
- Henken AM, Schaarsberg AMJG, Nieuwland MGB (1983) The effect of environmental-temperature on immune-response and metabolism of the young chicken 3: Effect of environmental-temperature on the humoral immune-response following injection of sheep red-blood-cells. *Poult Sci* 62:51–58
- Hochachka W, Smith JNM (1991) Determinants and consequences of nestling condition in song sparrows. *J Anim Ecol* 60:995–1008
- Hoi-Leitner M, Romero-Pujante M, Hoi H, Pavlova A (2001) Food availability and immune capacity in serin nestlings. *Behav Ecol Sociobiol* 49:333–339
- John JL (1994) The avian spleen: a neglected organ. *Q Rev Biol* 69:327–351
- John JL (1995) Parasites and the avian spleen: Helminths. *Biol J Linnean Soc* 54:87–106
- Kirkley JS, Gessaman JA (1990) Ontogeny of thermoregulation in red-tailed hawks and Swainson's hawks. *Wilson Bull* 102:71–83
- Klasing KC (1998) Nutritional modulation of resistance to infectious disease. *Poult Sci* 77:1119–1125
- Liker A, Márkus M, Vozár A, Zemankovics E, Rózsa L (2001) Distribution of *Carnus hemapterus* in a starling colony. *Can J Zool* 79:574–580
- Linden M, Gustafsson L, Part T (1992) Selection on fledging mass in the collared flycatcher and the great tit. *Ecology* 73:336–343
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary
- Lochmiller RL, Vestey MR, Boren JC (1993) Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk* 110:503–510
- Miller L, Qureshi MA (1992) Induction of heat-shock proteins and phagocytic function of chicken macrophages following in vitro heat exposure. *Vet Immunol Immunopathol* 30:179–192
- Møller AP, Erritzøe J (2000) Predation against birds with low immunocompetence. *Oecologia* 122:500–504
- Møller AP, Sorci G, Erritzøe J (1998a) Host immune function and sexual selection in birds. *J Evol Biol* 11:703–719
- Møller AP, Sorci G, Erritzøe J, Mavarez J (1998b) Condition, disease, and immune defence. *Oikos* 83:301–306
- Perrins CM (1964) Survival of young swifts in relation to brood size. *Nature* 201:1147
- 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
- Smith HG (2004) Selection for synchronous breeding in the European starling. *Oikos* 105:301–311
- Smith KG, Hunt JL (2004) On the use of the avian spleen as a measure of avian immune strength. *Oecologia* 138:28–31
- Smits JE, Williams TD (1999) Validation of ecotoxicology techniques in passerine chicks exposed to oil sands tailings water. *Ecotoxicol Environ Saf* 44:105–112
- Taylor RLJ, Cotter PF, Wing TL, Briles WE (1987) Major histocompatibility B complex and sex effects on the phytohaemagglutinin wattle response. *Anim Genet* 18:343–350
- Tella JL, Bortolotti GR, Dawson RD, Forero MG (2000) The T-cell-mediated immune response of fledgling American kestrels are positively correlated with parental clutch size. *Proc R Soc Lond B* 267:891–895
- Thompson CF, Flux JEC (1988) Body mass and lipid content at nest-leaving of European starlings in New Zealand. *Ornis Scandinavica* 19:1–6
- Thompson CF, Flux JEC, Tetzlaff VT (1993) The heaviest nestlings are not necessarily the fattest nestlings. *J Field Ornithol* 64:426–432
- Visser GH (1998) Development of temperature regulation. In: Starck JM, Ricklefs RE (eds) *Avian growth and development: Evolution within the altricial-precocial spectrum*. Oxford Ornithology Series, Oxford University Press, Oxford, pp 117–156
- Warner CM, Meeker DL, Rothschild MF (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