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Immune function and survival of great tit nestlings in relation to growth conditions

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Abstract Life history theory predicts a trade-off between number and quality of offspring. Reduced quality with increasing brood size may arise from a decrease in body condition or in immunocompetence that would be important in fighting off virulent parasites by immunologically naive offspring. We tested the effect of rearing conditions on immune function of nestling great tits (*Parus major*) by reducing or increasing broods by two hatchlings. In the middle of the nestling period (on day 8), nestlings from enlarged broods developed lower T cell responses [as measured from the cutaneous swelling reaction to injection with phytohaemagglutinin (PHA)] and tended to have lower total leukocyte and lymphocyte concentrations in their peripheral blood than nestlings from reduced broods. Brood size manipulation affected the PHA response of nestlings most strongly in small clutches, suggesting that nestling immune function was dependent on their parents' condition, as estimated by original clutch size. Intra-brood differences in nestling mortality were unrelated to immune parameters, but nestlings in broods without mortality had a stronger PHA response, higher concentration of lymphocytes and higher body mass on day 15 than nestlings in broods with mortality. These results support the prediction that the immune function of altricial birds is affected by rearing conditions, and that growth and immune parameters are related to inter-brood differences in nestling survival.

Key words Brood size manipulation · Nestling immunocompetence · Nestling survival · *Parus major* · Phytohaemagglutinin test

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Introduction

Life history theory deals with the evolution of breeding date, clutch size and similar traits, and how these have coevolved to a suite of traits that maximise fitness in a given environment with given ecological conditions (Roff 1992; Stearns 1992). Reproductive patterns of iteroparous organisms are shaped by two major life history trade-offs: one between current and future reproduction and one between offspring number and quality. Recently, animal ecologists have suggested that immune function may potentially play an important role in mediating both trade-offs (e.g. Sheldon and Verhulst 1996; Møller and Saino 1998; Saino et al. 1998). This approach has challenged a traditional view (e.g. Perrins 1965; Nur 1984; Smith et al. 1989; Tinbergen and Boerlijst 1990; Lindén et al. 1992) that for altricial birds, the advantage of having relatively few nestlings stems from larger body mass of individual fledglings that increase their probability of survival during the critical post-fledging period. Hence, the question about the relative importance of these two pathways arises: if the amount of resources available for nestlings is scarce, should they sacrifice both immune function and growth, or should they sacrifice one over the other?

Current evidence concerning resource limitation of immune function in nestlings originates from brood size manipulation experiments in barn swallows *Hirundo rustica* (Saino et al. 1997a, 1998) and collared flycatchers, *Ficedula albicollis* (Nordling 1998) in which nestlings from enlarged broods (which received less food per capita) developed reduced immunocompetence compared to nestlings raised in control and experimentally reduced

broods. Moreover, the latter study, as well as that of Christe et al. (1998) on house martins *Delichon urbica*, also demonstrated positive correlations between nestling survival and their immunocompetence. Although not providing unambiguous proof that immunocompetence is more important for nestling survival than body condition, these results suggest increased fitness of immunocompetent altricial nestling birds. The generality of this conclusion, however, awaits further research involving different species and a wider range of immune tests and measurements of the health status of nestlings.

Consequently, the aim of the present study was to test for the relationships between immune function, body mass and growth conditions, but also the relationship between both immune and growth parameters and survival of nestlings of the great tit (*Parus major*), a small (ca 19 g) hole-nesting passerine bird. We manipulated nestling growth conditions by increasing or reducing broods by two nestlings on the second day after hatching to test whether nestling immune function is resource limited. The underlying assumption of this experiment, namely that clutch size is individually adjusted to the amount of resources that parents can provide to their nestlings, is based on experimental evidence for the almost ubiquitous effects of brood size manipulation on nestling quality (reviewed in Lessells 1991; Roff 1992; Stearns 1992). To assess the immune function of nestlings, we estimated one component of immunocompetence – T-cell-mediated immune responsiveness to challenge with the plant lectin phytohaemagglutinin (PHA) – by measuring the magnitude of the cutaneous swelling in response to injection of the novel antigen (see e.g. McCorkle et al. 1980; Saino et al. 1997a). Furthermore, we recorded body mass and leukocyte profiles and concentrations in the peripheral blood of nestlings.

We predicted that if immune function of nestlings is resource limited, then nestlings from enlarged broods should show a weaker immune response against PHA and have a lower concentration of lymphocytes than nestlings from reduced broods. If poor growth conditions affect leukocyte indicators of stress (e.g. Maxwell 1993), we expected elevated heterophil/lymphocyte ratios and peripheral heterophilia (which is also symptomatic of infectious challenge) in nestlings reared in enlarged broods. Additionally, we compared the immune and the growth parameters of surviving and non-surviving nestlings within broods, with the prediction that non-survivors should reveal symptoms of immune suppression, and tested whether immune function was related to the general phenotypic quality of the entire brood by comparing the condition of nestlings from broods with and without mortality.

Materials and methods

General procedures and measurement of immune parameters

The study was carried out in spring 1998 in an urban great tit population breeding in nest boxes in Tartu, south-east Estonia (58°22’ N, 26°43’ E). At the age of 8 days, approximately half of the nestlings in 27 experimental broods were injected intradermally in the wing web (the patagium) with 0.2 mg of PHA (Sigma, L-8754) in 0.04 ml isotonic saline (the antigen injection), while the left wing web was injected with the same amount of saline only (a control injection). The thickness of the skin comprising the wing webs was measured immediately before and 24 h after injection in inoculated sites using a pressure-sensitive calliper, a so-called spessimeter (Alpa, Milano, code SM112), with an accuracy of 0.01 mm. To express the reaction to PHA while controlling for the effect of injection per se and thickening due to saline injection, we calculated the difference between the change in thickness of the right PHA-injected wing web (thickness 24 h after injection minus thickness before injection) and the corresponding change in thickness of the left wing web, only injected with saline. This procedure, as well as the type of spessimeter and amounts of PHA and saline were identical to those used by Saino et al. (1997a) in their study of barn swallows. The thickness of the wing web was measured three times both before and 24 h after PHA injection, and the average of these three measurements was used in the calculations. We found no systematic differences between the wing web indices calculated on the basis of consecutive measurements [average difference between the wing web indices calculated on the basis of first and third measurement=0.008±0.147 (SD) mm, t_{102}=0.53, P=0.597]. The repeatability (Lessells and Boag 1987) of the wing web index was 0.91 ($F_{102,308}$=29.9, P<0.001).

Immediately before and a week after treatment with PHA (at the age of 15 days when great tit nestlings have attained adult size and are ready to leave the nest), nestlings were weighed and blood sampled for measurement of leukocyte parameters. An elevated total leukocyte count is characteristic for inflammatory processes in response to microbial and macroparasite infections (e.g. Dein 1986), and is usually accounted for by an increase in the number of the two most numerous leukocyte types, namely heterophils and lymphocytes. Heterophils are non-specific phagocytosing cells that enter tissues during an inflammatory response. Lymphocytes elicit pathogen-specific immune responses. T lymphocytes (which comprise the majority of circulating lymphocytes) play a key role in cell-mediated immunity, while B lymphocytes that produce immunoglobulins are primarily responsible for antibody-mediated or humoral immunity. The interpretation of variation in lymphocyte counts is, however, complicated since relatively low concentration of lymphocytes may signal either lack of parasitic infection (see e.g. Ots and Hõrak 1998) or indicate stress-induced immunosuppression (e.g. Hõrak et al. 1998), which is expected to increase susceptibility to viral infections (Siegel 1985; Fitzgerald 1988). An index comprising the relative abundance of both lymphocytes and heterophils is the heterophil/lymphocyte (H/L) ratio, high values of which are traditionally regarded as indicative of stress in poultry studies (e.g. Gross and Siegel 1983; Maxwell 1993), but also in free-living passerine birds (e.g. Ots and Hõrak 1996).

All leukocyte parameters were estimated from blood samples collected (ca 5 µl on day 8 and ca 150 µl on day 15) from the tarsal or brachial vein. A drop of blood was smeared on two individually marked microscope slides, which were air-dried, fixed in absolute methanol, and stained with azure-eosin. The proportion of different types of leukocytes was assessed on the basis of an examination of a total of 100 leukocytes under ×1000 magnification under oil immersion. Estimates of the total white blood cell count (WBC) were obtained by counting the number of leukocytes per approximately 10,000 erythrocytes. Differential leukocyte counts were obtained by multiplying their proportions with WBC. The repeatabilities of leukocyte counts obtained by this method were found to be reasonably high and significant (Ots et al. 1998). Similar methods for estimating leukocyte concentrations have also been used by e.g. Dufva and Allander (1995), Saino et al. (1995, 1997b) and Saino and Møller (1996). The investigators examining blood samples were unaware of the phenotypic values of the birds. Nestlings were weighed on days 8 and 15 after hatching using a Pesola spring balance with a precision of 0.1 g.

Table 1 Comparison of nestling parameters in experimentally reduced (n=13) and enlarged (n=12) great tit broods at the age of 8 days. All means are calculated for the same set of nestlings. Values are means with the SD in parentheses (*PHA* phytohaemagglutinin, *WBC* white blood cell count, *H* heterophils, *L* lymphocytes)

Trait	Reduced broods	Enlarged broods	P (F)	R^2
Brood size	7.62 (1.26)	10.66 (1.50)	<0.001 (30.6)	0.57
Nestling body mass (g)	12.1 (1.3)	11.3 (1.7)	0.156 (2.1)	0.09
PHA response (mm)	0.94 (0.28)	0.72 (0.25)	0.050 (4.3)	0.16
log(WBC/10^4 erythrocytes)	3.71 (0.28)	3.51 (0.22)	0.061 (3.9)	0.14
log(lymphocytes/10^4 erythrocytes)	3.44 (0.27)	3.25 (0.23)	0.088 (3.2)	0.12
log(heterophils/10^4 erythrocytes)	2.15 (0.42)	1.92 (0.33)	0.158 (2.1)	0.08
log (H/L)	–1.29 (0.36)	–1.33 (0.31)	0.763 (0.1)	0.00

Brood size manipulation and data analysis

For the brood size manipulations, two 2-day-old (day 0=day of hatching) nestlings were moved from 'reduced' nests to 'enlarged' nests with similar hatching date (±1 day) and clutch size (±1 egg). However, in approximately half of the experimental dyads, one brood from the pair failed to survive until the 8th day of the nestling period, which resulted in a non-significant tendency ($F_{1,24}$=1.7, P=0.206) for the original clutch size to be larger in remaining reduced broods (9.9±1.4) than in remaining enlarged broods (9.1±1.8). To account for the possible differences due to original clutch size, we included original clutch size as a covariate in all models and checked if this affected the significance level of experimental effect on growth and immune parameters. Since hatching dates of remaining experimental brood categories did not differ (average difference=0.15 days, $F_{1,24}$=0.0, P=0.867), and since the effect of hatching date and its interaction with brood size manipulation were non-significant, we did not retain hatching date as a covariate in the models. All experimental broods were within the size range found naturally in the population. Sample sizes of parameters measured on day 8 and 15 differed because three broods (two enlarged and one reduced) were depredated after day 8.

We used two approaches when analysing the relationship between nestling parameters and survival. First, we examined the difference between surviving and non-surviving siblings by testing whether the average within-brood difference of surviving and non-surviving nestlings deviated significantly from 0. This analysis could be performed only on parameters measured on day 8 since the number of broods for which leukocyte parameters had been measured on day 15 and partial nestling mortality occurred after day 15 was too low (4) for statistical analysis. Second, because this analysis excludes all broods where all or none of the nestlings died, we also compared growth and immune parameters of broods with and without nestling mortality (excluding depredated broods). Additionally, we performed a multiple logistic regression analysis, using the proportion of surviving nestlings as a binomial dependent variable in order to test for the simultaneous effects of immune and growth parameters on nestling survival (SAS GENMOD procedure with binomial error distribution and logit link function). The fit of the models with the data was satisfactory as no overdispersion was indicated (scaled deviance/df ratio<2). In the analysis of the relationship between condition indices and nestling survival, broods not subjected to PHA challenge were also included in order to increase sample size. All trait values (except morphological parameters that were normally distributed) were log-transformed, which allowed us to use parametric statistical procedures. All analyses were based on brood mean values for all nestling parameters, unless stated otherwise. All statistical tests were two-tailed. 1998 was a 'typical' year in terms of breeding success, as the fledging success (number of fledglings/clutch size) in unmanipulated broods did not differ significantly from that of the average of 11 previous years [0.62±0.32 (SD) in 1998 vs 0.62±0.32 in previous years; $t_{36,516}$=0.53, P=0.909]. Average clutch size in 1998 (9.39±1.44) was, however, higher than the average of 11 previous years (9.00±1.60; $t_{79,739}$=2.06, P=0.039).

Results

None of the leukocytic parameters of individual nestlings, measured on day 8, correlated significantly with magnitude of the PHA response (r=0.04–0.12, P=0.26–0.67, n=90). However, nestlings that mounted a stronger swelling reaction against PHA on day 8 had higher lymphocyte haemoconcentrations (r=0.41, P<0.001, n=67) and higher total leukocyte counts (r=0.40, P<0.001, n=67) on day 15, while the heterophile count and H/L ratio on day 15 did not correlate with the magnitude of the PHA response (r=0.04–0.18, P>0.13, n=67).

Enlarged broods contained on average three nestlings more than reduced broods by day 8 of the nestling period (Table 1). Nestlings from enlarged broods mounted a significantly weaker PHA response and had marginally lower total leukocyte and lymphocyte concentrations in their peripheral blood than nestlings from reduced broods. The differences in WBC and lymphocyte counts became significant when two additional broods with two unhatched eggs were included in the category of reduced broods ($F_{1,26}$=5.5, P=0.028 for WBC; $F_{1,26}$=4.7, P=0.040 for lymphocyte count). However, when the original clutch size was added as a covariate to the model, the effect of brood size manipulation on WBC and lympho-

Table 2 Mean intra-brood differences (mean of survivors–mean of non-survivors) in condition indices of surviving and non-surviving 8-day-old great tit nestlings. Significance levels from paired t-tests, under the null hypothesis that the mean within-brood difference equals 0. n=13 broods; all means are calculated for the same set of nestlings. Minimum detectable difference calculated for 90% test power for two-tailed test with significance level of 5%

Trait	Difference (SD)	Minimum detectable difference	P
Nestling body mass (g)	1.4 (1.9)	1.7	0.019
log(WBC/10^4 erythrocytes)	0.12 (0.41)	0.37	0.300
log(lymphocytes/10^4 erythrocytes)	0.16 (0.46)	0.41	0.239
log (heterophils/10^4 erythrocytes)	0.10 (0.61)	0.66	0.578
log (H/L)	–0.06 (0.70)	0.61	0.763

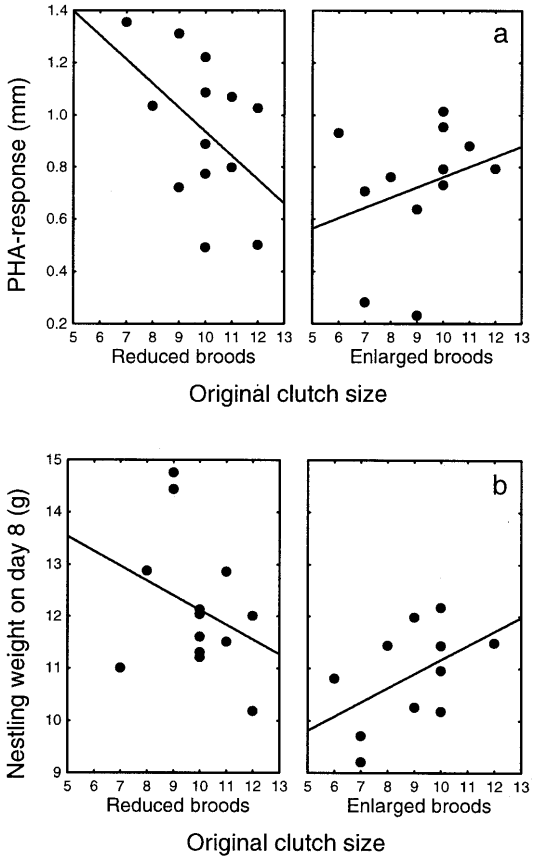

Fig. 1 The relationship between clutch size and phytohaemagglutinin response (mm) (**a**) and nestling body mass (g) (**b**) for great tit broods that were reduced or enlarged by two nestlings. The *lines* are the linear regressionlines

cytes vanished ($F_{1,26}$=3.5, P=0.073 for WBC; $F_{1,26}$=2.9, P=0.101 for lymphocyte count), while a significant positive effect of clutch size on leukocyte counts emerged ($F_{1,26}$=4.8, P=0.038 for WBC; $F_{1,26}$=4.5, P=0.045 for lymphocyte count). Unlike the results with leukocyte counts, the effect of brood size manipulation on PHA response remained significant ($F_{1,24}$=5.4, P=0.030) in a model adjusting for the main effects of clutch size ($F_{1,24}$=0.6, P=0.432) and the clutch size×manipulation interaction term ($F_{1,24}$=4.0, P=0.059). Heterophil concentration, H/L ratio and body mass of nestlings did not differ significantly between reduced and enlarged broods in models with the brood size manipulation as a single explanatory variable (Table 1). However, the effect of brood size manipulation on nestling body mass became significant ($F_{1,24}$=5.6, P=0.028) in a model adjusting for the main effects of clutch size ($F_{1,24}$=0.4, P=0.548) and the clutch size×manipulation interaction term ($F_{1,24}$=4.0, P=0.041). For leukocyte counts, no significant manipulation×clutch size interaction terms were detected (all P values>0.5).

The effect of brood size manipulation on PHA response and body mass depended on original clutch size (Fig. 1); both PHA response and body mass decreased with increasing clutch size in reduced broods, but increased with increasing clutch size in enlarged broods.

To test whether the effect of brood size manipulation on PHA response can be explained by effects of experimental treatment on nestling body mass, we added average nestling mass per brood as a covariate in the ANCOVA model with the effects manipulation, clutch size and clutch size× manipulation. In this model, both the effect of brood size manipulation on PHA response ($F_{1,24}$=3.2, P=0.090) and the effect of nestling body mass on PHA response ($F_{1,24}$=0.3, P=0.580) were non-significant. None of the examined parameters was significantly affected by brood size manipulation on day 15 of the nestling period, irrespectively of whether or not the original clutch size was included in the models as a covariate (P=0.12–0.81; 9 enlarged and 11 reduced broods).

Nestlings that died between day 8 and 15 of the nestling period weighed significantly less than their siblings on day 8, while none of the leukocyte parameters was significantly related to intra-brood differences in survival

Table 3 Comparison of nestling parameters in great tit broods without nestling mortality (n=11) and broods where at least one nestling died before fledging (n=20). All means (except for PHA response) are calculated for the same set of nestlings. Values are means with the SD in parentheses. Significance levels from t-test. Minimum detectable difference calculated for 90% test power for two-tailed test with significance level of 5%

Trait	Without mortality	With mortality	Minimum detectable difference	P
Brood size on day 8	7.73 (1.79)	8.95 (2.28)	2.41	0.136
Brood size on day 15	7.73 (1.79)	6.90 (2.88)	2.72	0.396
Nestling body mass on day 8 (g)	12.1 (1.1)	11.7 (1.8)	1.7	0.552
Nestling body mass on day 15 (g)	16.7 (0.7)	15.2 (1.7)	1.4	0.002
PHA response (mm)[a]	1.00 (0.24)	0.71 (0.26)	0.36	0.018
log(WBC/10^4 erythrocytes) on day 8	3.50 (0.27)	3.63 (0.25)	0.32	0.175
log(WBC/10^4 erythrocytes) on day 15	3.71 (0.16)	3.58 (0.34)	0.29	0.146
log(lymphocytes/10^4 erythrocytes) on day 8	3.22 (0.26)	3.35 (0.24)	0.31	0.178
log(lymphocytes/10^4 erythrocytes) on day 15	3.45 (0.16)	3.26 (0.35)	0.30	0.046
log(heterophils/10^4 erythrocytes) on day 8	1.96 (0.37)	2.11 (0.32)	0.43	0.237
log(heterophils/10^4 erythrocytes) on day 15	2.05 (0.46)	2.06 (0.59)	0.62	0.973
log(H/L) on day 8	–1.26 (0.26)	–1.23 (0.21)	0.30	0.805
log(H/L) on day 15	–1.40 (0.50)	–1.20 (0.58)	0.64	0.348

[a] Eight broods without and 14 with nestling mortality

(Table 2). We compared broods with no nestling mortality with those in which at least one nestling died before fledging to examine whether growth and immune parameters were related to the phenotypic quality of the entire brood (Table 3). Nestlings from broods with no mortality had a stronger PHA response, larger body mass on day 15 and higher lymphocyte count on day 15 than nestlings from broods where at least one young died before fledging. To compare the relative importance of lymphocyte count and body mass for nestling survival, we performed a multiple logistic regression analysis, using the proportion of surviving nestlings as a binomial dependent variable and average lymphocyte count and body mass per brood (both measured on day 15) as predictors of nestling survival. When entered simultaneously into the model, the effect of body mass was significant ($\chi^2_{1,28}$=8.63, P=0.003), while the effect of lymphocyte count was not ($\chi^2_{1,28}$=1.23, P=0.268).

Discussion

Leukocytic immune parameters measured on day 8 did not correlate with subsequent immune response to PHA, indicating that these indices reflected independent parameters of the immune system of great tit nestlings. The result that nestlings that had mounted a stronger immune response against PHA on day 8 had higher lymphocyte (and hence total leukocyte) haemoconcentrations on day 15 suggests that high pre-fledging lymphocyte counts are characteristic of nestlings with well-developed T-cell-mediated immune responsiveness.

Brood size manipulation affected immune responses of nestling great tits as demonstrated by a measure of immunocompetence (PHA response). Nestlings from enlarged broods were less capable of mounting a cutaneous swelling reaction against a novel antigen and also tended to have lower concentrations of circulating leukocytes in their peripheral blood at the age of 8 days. The simplest explanation of this effect of brood size manipulation would be that the immune function of nestlings is directly resource limited. An alternative, although not mutually exclusive possibility would be that larger broods may attract more parasites, e.g. because parents are visiting large broods more frequently, increasing the transmission rates, or just because larger broods smell more and parasites can find them more easily. In the latter case, we would have expected a direct effect of original clutch size on nestling immune parameters. Such an effect was not present for the PHA response, while the lymphocyte count and WBC indeed increased with brood size.

The effect of brood size manipulation on PHA response depended on original clutch size: while the measure of the T cell response decreased with clutch size in reduced broods, the T cell response increased with clutch size in enlarged broods (Fig. 1). This finding implies that experimental brood reduction particularly favours pairs with small clutches, while brood enlargement is particularly detrimental to nestlings in small broods. The proximate explanation for this effect is that proportionally greater modification of brood size induced greater differences for nestling immunocompetence. Additionally, this result is consistent with clutch size being condition dependent; if so, and large clutches are laid by females in prime condition, then we should expect experimental reduction of brood size to particularly favour pairs in poor condition, while brood size enlargement should have the opposite effect. The mechanism giving rise to this effect could be (1) females in better condition laying eggs of better quality containing more immunoglobulins, carotenoids or yolk, (2) pairs in better condition providing food of superior quality or quantity for their offspring, and/or (3) such pairs having condition-dependent genetic viability associated with immune responsiveness.

Brood size manipulation did not affect the H/L ratio of nestlings, which suggests that impairment of growth conditions does not induce a stress syndrome. Lack of heterophilia in nestlings from enlarged broods suggests that experimental manipulation of growth conditions did not affect the susceptibility of nestlings to infections. Notably, none of the measures of nestling condition affected by the brood size manipulation in the middle of the nestling period differed significantly between reduced and enlarged broods at day 15. This was probably caused by reduced differences in growth conditions between experimental brood categories by day 15, because brood size of enlarged and reduced broods only differed significantly on day 8, but not on day 15.

The effects of brood size manipulation on a measure of immunocompetence (PHA response) and on leukocytes support the idea that nestling immune function is sensitive to growth conditions. This is an important assumption of the hypothesis that the trade-off between number and quality of offspring in altricial birds is mediated by immunosuppression. Thus, our results are similar to those of Saino et al. (1997a) showing that barn swallow nestlings reared in enlarged broods had lower T cell responses than those reared in reduced broods. Similarly, using a different immunoassay (measuring clearance rate of a subcutaneously applied antigen), Nordling (1998) demonstrated a negative correlation between experimental brood size and immunocompetence of nestling collared flycatchers.

Immune parameters did not predict survival of great tit nestlings in the nest, as shown by intra-brood differences in nestling condition. Although the sample size was insufficient for a within-brood comparison of the PHA response of surviving and non-surviving siblings, leukocyte parameters sensitive to brood size manipulation (Table 1) did not differ significantly between surviving and non-surviving siblings (Table 2). This result could perhaps be explained by a low power of the statistical test due to small sample size (13 broods). However, it should be noted that with the same sample, surviving nestlings had significantly larger body mass at age 8 days than their non-surviving siblings. Hence, survival of individual great tit nestlings within broods seems to depend more on body mass than on the number of circu-

lating immune cells. This result differs from those of Christe et al. (1998), who showed that non-surviving house martin nestlings had lower immunocompetence than their surviving siblings. This difference could be explained by the fact that the leukocyte counts and PHA response in house martins were recorded at an older age (day 15 vs day 8 in the current study), when the immune system of nestlings is more mature, and hence more likely to reflect individual differences in fitness. Alternatively, the ectoparasite burden may have differed between the two studies.

Does the lack of covariation between immune parameters and survival within broods imply that immune function is of little importance for nestling survival in great tits? Such a conclusion would be premature since the test only relates to broods in which at least one nestling died after day 8 of the nestling period. Several parents managed to fledge all their nestlings, suggesting differences in phenotypic quality among broods. Indeed, a comparison between broods with and without nestling mortality revealed that nestlings in broods with no mortality had a stronger PHA response, higher lymphocyte counts and larger body mass on day 15. Since broods without nestling mortality have superior production of recruits in this particular great tit population (Hõrak 1995), one might conclude that variation in immune function, at least among broods, can contribute to fitness. However, our data do not allow the conclusion that immune parameters are more important than body mass, since the multiple logistic regression indicated that the effect of body mass on nestling survival cancelled that of lymphocyte count. This analysis only deals with survival between day 15 of the nestling period and fledging, and does not exclude the possibility that factors other than body mass could be important for post-fledging survival (the period between fledging and breeding being a particularly vulnerable one for great tits: van Noordwijk and van Balen 1988; McCleery and Perrins 1988).

In conclusion, our findings support the prediction that immune function of altricial birds is affected by their growth conditions, and also that differences in nestling survival among broods are related to immunocompetence. However, we could not distinguish between the relative importance of nutritional state and immunocompetence for nestling survival. This question needs to be addressed in further studies manipulating immunocompetence while experimentally controlling for the effect of nestling body mass.

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