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Immunocompetence of nestling great tits in relation to rearing environment and parentage

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Theoretical models of host–parasite coevolution assume a partially genetic basis to the variability in susceptibility to parasites among hosts, for instance as a result of genetic variation in immune function. However, few empirical data exist for free-living vertebrate hosts to support this presumption. In a cross-fostering experiment with nestling great tits, by comparing nestlings of the same origin we investigated (i) the variance in host resistance against an ectoparasite due to a common genetic origin, (ii) the effect of ectoparasite infestation on cell-mediated immunity and (iii) the variance in cell-mediated immunity due to a common genetic origin. Ectoparasitic hen fleas can impair the growth of nestling great tits and nestling growth was therefore taken as a measure of host susceptibility. A common origin did not account for a significant part of the variation in host susceptibility to fleas. There was no significant overall effect of fleas on nestling growth or cell-mediated immunity, as assessed by a cutaneous hypersensitivity response. A common rearing environment explained a significant part of the variation in cell-mediated immunity among nestlings, mainly through its effect on nestling body mass. The variation in cell-mediated immunity was also related to a common origin. However, the origin-related variation in body mass did not account for the origin-related differences in cell-mediated immunity. The results of the present study thus suggest heritable variation in cell-mediated immunity among nestling great tits.

Keywords: nestling body mass; *Ceratophyllus gallinae*; cross-fostering; great tits; cell-mediated immunity; *Parus major*

1. INTRODUCTION

Studies of natural host–parasite systems have generally shown considerable variation in the prevalence of parasites between individual hosts (reviewed by Goater & Holmes 1997). Such phenotypic variation in parasite load might be caused by interindividual differences in host behaviour as well as physiology. The variability in the susceptibility or resistance of hosts to parasites might be partly environmental, but theoretical models of host–parasite coevolution generally assume a genetic basis as well. This notion concerns, for example, models for the evolution of host sexual reproduction (Hamilton 1980) and sexual selection (Hamilton & Zuk 1982). Life history traits such as those involved in host defence against parasites can only evolve if they show additive genetic variance, that is if the variation in the trait is at least partly heritable (Falconer & Mackay 1996). Therefore, to understand the evolution of host–parasite interactions, the genetic and environmental control of host resistance or susceptibility to parasites needs to be investigated.

The most important physiological defence that vertebrate hosts have evolved against parasites is the acquired immune system (Roitt *et al*. 1996). Several studies have shown that the expression of an acquired immune response may effectively control parasite loads (Baron & Weintraub 1987; Wakelin & Apanius 1997). Induced responses (of which immunological responses are an important component) can also increase host tolerance towards ectoparasites (Heeb *et al*. 1998). The variation in acquired immune responses can be partly environmental, mainly through the effect of food quality or quantity on nutritional condition (Gershwin *et al*. 1985; Cook 1991). Its expression also has a genetic basis, as shown in studies on laboratory and domestic animals (Wakelin & Blackwell 1988; Wakelin & Apanius 1997). Detailed analyses of the prevalence of parasites on domestic animals have been successful in partitioning the phenotypic variance in parasite load by degree of relatedness (Goater & Holmes 1997). However, heritability estimates under such controlled conditions might be a poor predictor of the expression of genetic variation in the field (Sorci *et al*. 1997). In general, heritability measured under laboratory conditions overestimates the amount of genetic variation expressed in the field, where the variation in environmental factors might contribute significantly to the total phenotypic variance in a trait (Hoffmann & Merilä 1999).

Few studies have examined the genetic variability in parasite resistance and immunocompetence in natural host populations (reviewed by Read *et al*. 1995; Sorci *et al*. 1997; Wakelin & Apanius 1997). In birds, evidence of heritable variation in host susceptibility has been confined to two studies on the prevalence of ectoparasites in natural populations. In a correlational study, Boulinier *et al*. (1997) found a positive relationship for tick load between parents and offspring. Strong support comes from Møller (1990), who demonstrated in a cross-fostering experiment that mite populations on nestling barn swallows (*Hirundo*

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Received 26 July 1999 *Accepted* 19 August 1999

rustica) were partly determined by sibling relationships. Significant additive genetic variation in cell-mediated immunity was shown in nestling barn swallows by Saino *et al*. (1997*a*).

The present study addressed the following three points. First, we investigated the potential for genetic variation in susceptibility to the ectoparasitic hen flea among nestling great tits (*Parus major*). Great tits are a usual host of the hen flea (*Ceratophyllus gallinae*; Tripet & Richner 1997) and flea infestation has repeatedly been shown to reduce nestling growth and condition (Richner 1998; Heeb *et al*. 1999). However, it is currently unknown whether factors related to the genetic origin of nestlings produce variation in susceptibility to hen fleas. We therefore performed a cross-fostering experiment and compared the relative growth of full sibs reared in flea-infested and -uninfested nests, respectively.

Second, we investigated the effect of ectoparasite infestation on one component of immunocompetence, i.e. cell-mediated immunity, as reflected in cutaneous hypersensitivity responses to an injection of phytohaemagglutinin (PHA; Lochmiller *et al*. 1993; Roitt *et al*. 1996; Saino *et al*. 1997*a*). Such an analysis might indicate whether ectoparasite infestation potentially alters the host's susceptibility to other pathogens. There are several not mutually exclusive predictions about the effect of an ectoparasite on cell-mediated immunity. Experimental feeding schedules have shown that young in poor nutritional condition generally show weaker cell-mediated immunity than those in superior condition (Lochmiller *et al*. 1993; Saino *et al*. 1997*a*). Therefore, the detrimental effects of hen flea infestation on nestling condition might indirectly result in reduced cell-mediated immunity. A lower response to PHA might also result if flea infestation mainly challenges other parts of the immune system specifically, causing nestlings to invest less in cell-mediated immunity. Alternatively, a higher response to PHA could result from flea infestation. Host immune reactions against ectoparasitic arthropods occur at the cutaneous interface and cell-mediated responses are key regulatory elements and potentially important effectors of host immunological protection (Wikel 1982; Wikel *et al*. 1996). To counter the detrimental effects of hen fleas, nestlings may therefore enhance their cell-mediated immune function leading to a higher cutaneous immune response to PHA.

Third, we investigated the variance in cell-mediated immunity due to a common genetic origin. Special attention was paid to investigation of nestling growth, since cross-fostering studies in great tits (Van Noordwijk *et al*. 1988; Gebhardt-Henrich & Van Noordwijk 1991) and other bird species (Price 1991; Merilä 1997) have indicated origin-related variation in nestling growth, which may in turn induce origin-related variation in immunocompetence. Therefore, using our cross-fostering design, we investigated to what extent the differences in immunocompetence within and between sibling groups were indirectly explained by the effects of a common origin and common rearing environment on nestling body mass.

2. MATERIAL AND METHODS

(a) *Study area and study species*

The experiment took place in spring 1997 in Bremgartenwald near Bern, Switzerland, where a great tit population breeding in nest-boxes has been studied for several years. Nests were regularly visited from the start of the breeding season onwards to determine the start of egg laying, clutch size, hatching date and fledging date. In great tits, hatching is asynchronous within broods, usually taking place over a period of one to two days. The hatching date was defined as the day on which at least half of the eggs hatched. Nestling body mass was measured on day 1 after hatching as a measure of initial size at cross-fostering and on day 12, i.e. at the time when cell-mediated immunity was assessed. Body mass was measured to a precision of 0.1 g using an electronic Sartorius balance.

(b) *Experimental procedures*

To investigate the significance of rearing environment and parentage on similarity in immunocompetence and body mass among nestlings, we started with 423 chicks from 61 different nests of origins (hereafter referred to as the origin). Cross-fostering took place on the first day (day 1) after hatching between sets ($n = 13$) of four ($n = 4$) or five ($n = 9$) nests, depending on the number of broods of the same age available on a certain date. We aimed to maximize the number of young cross-fostered into foreign nests. Within a cross-foster group, broods of different origin were ordered according to mean body mass, while individual nestlings were ranked according to body mass within their brood of origin. Nestlings of the same origin were then sequentially distributed over foreign broods according to their rank within their brood of origin and the rank between potential foster broods. The nestling of a given origin ranked 1 was assigned to the heaviest foster brood, nestling rank 2 to the second heaviest foreign brood and so on until all foreign broods had received one nestling of each origin. This procedure was then repeated until the nestlings of each remaining origin were appointed to a foreign brood. By cross-fostering young between broods according to body mass we intended to keep the initial within-brood variance in body mass close to the values occurring under natural conditions, while nestlings of the same origin were adequately distributed with respect to rank within the newly created foster brood. To avoid an effect on brood size, no further nestlings were added once the newly created foster brood had reached its original brood size. As a consequence, the lightest young of the largest broods within a cross-foster group could not be transferred to a foreign nest. These young ($n = 55$ from 34 different origins) remained in their home nest and were excluded from further analysis. Two flea-infested broods (see below), containing 16 chicks in total, completely failed, while partial brood mortality included 12 chicks. Analyses were performed on the remaining 340 chicks. All young were individually marked by clipping different combinations of down feathers and could thereby be identified up until day 9 after hatching, when banding with numbered aluminium rings took place.

To create standard differences in the ectoparasite level between foster broods, all nests were heat treated in a microwave appliance to eliminate nest-based ectoparasites (Richner *et al*. 1993) following cross-fostering on day 1. Within cross-foster groups of four broods, we then infested two randomly chosen foster broods with 40 adult hen fleas collected from nests infested in the previous year in the same local population. In cross-foster groups of five broods, three broods were experimentally infested. The remaining nests in a cross-foster group were not infested and remained untreated up until fledging. Natural reinfestation with hen fleas is low (Heeb *et al*. 1996). The combination of cross-fostering and partial flea infestation (non-infested

nests $n = 26$ and infested nests $n = 33$) allowed us to investigate whether the effect of hen fleas on nestling growth depended on origin. Such an interaction between flea treatment and origin would indicate an origin-related variation in susceptibility to hen fleas in our great tit population.

Our cross-fostering design did not allow us to discriminate between pre-hatching maternal effects (Heeb *et al*. 1998) and genetic variance in body mass or immunocompetence. In the remainder of this paper we therefore refer to a common origin effect to indicate the combination of genetic effects and maternal effects. Initial size differences between nestlings of different origin upon cross-fostering are in this respect of special concern, as differences between unrelated sib groups might be maintained or enhanced through sibling competition (Van Noordwijk 1988). Initial size differences in relation to origin indicate shared early environmental (up to day 1) or maternal effects and these could principally explain any association between origin and body mass or immunocompetence. The body mass of nestlings at cross-fostering was therefore measured and statistically controlled for in the data analyses.

(c) *Assessment of immunocompetence*

To resist infection by parasites and disease the immune system relies on three major components: phagocytosis, T-cell-mediated immunity and antibody response (Cheng & Lamont 1988). The present study deals with cell-mediated immunity and uses a delayed cutaneous hypersensitivity response as measure of T-cell reactivity. This was assessed by a subcutaneous injection of PHA-P (Sigma Chemicals, Division of Fluka Chemie AG, Switzerland) in the wing web, a thin layer of skin between the radius and humerus and measuring the swelling 24 h later (for a similar approach see Saino *et al*. (1997*a*) and Zuk & Johnsen (1998)). When the nestlings were 12 days of age, we injected one wing web with 0.1 mg of PHA dissolved in 0.02 ml of phosphate-buffered saline (PBS) while the other wing web was injected with PBS only. The thickness of the wing web was measured, prior to injection as well as 24 h (range ± 1 h) later to the nearest 0.01 mm with a micrometer, which discharged a constant pressure on the soft tissue (Mitotuyo, Schweiz AG, Switzerland, type 2046FB-60). However, upon measurement of the response, it took a considerable time before the measurement stabilized. To standardize the measurements, we took the thickness 5 s after applying the micrometer. The difference in the swelling of the wing web between the PHA-injected and PBS-injected sides was taken as a measure of the cell-mediated immune response. The accuracy of our measurement was assessed among a sample of 50 nestlings where the wing web swelling was measured three times in sequence. Due to the pressure of the micrometer, the wing web thickness declined with successive measurement. Controlling statistically for this gradual decline with successive measurement, the measures were highly repeatable ($r = 0.94$ and $p < 0.0001$). Similar high values for r were reported in the studies by Saino *et al*. (1997*a*) and Zuk & Johnsen (1998).

(d) *Statistical analysis*

To assess the effect of rearing environment and origin on wing web swelling and body mass among individual nestlings we fitted nested ANOVA models with JMP IN statistical software (Sall & Lehmann 1996). Since the numbers of young per family were unequal (unbalanced design), a random statement was used to generate random-model mean squares and error estimates for *F*-tests. Causal components of variance were estimated using the Satterthwaite approximation (Sall & Lehmann 1996). The use of restricted maximum likelihood in SAS (SAS Institute, Inc. 1988) produced similar estimates for the variance components (M. W. G. Brinkhof, unpublished data).

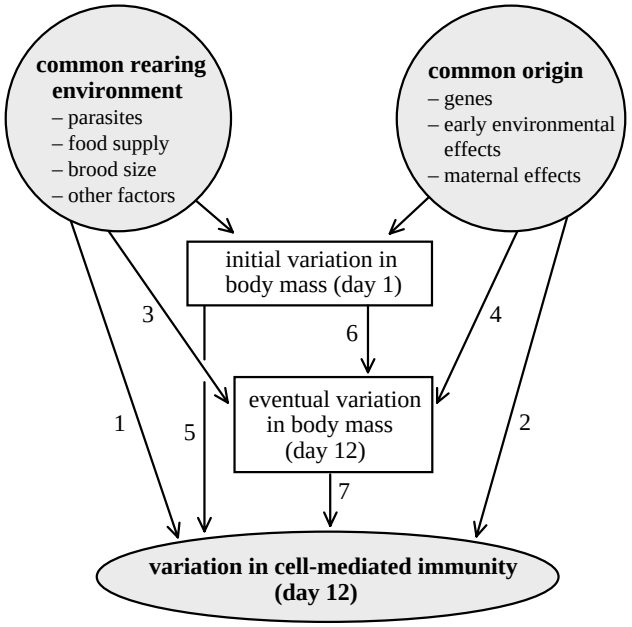

Figure 1. Scheme of hypothesized causes for the variation in cell-mediated immunity among individual nestlings. Nest of rearing-related variation in cell-mediated immunity (pathway 1) may result from local environmental factors such as food supply, brood size or parasite load. Common origin effects on cell-mediated immunity (pathway 2) may reflect genetic variance as well as non-genetic maternal effects or early developmental effects after hatching. The effects of a common rearing environment or common origin on the cell-mediated immune response may indirectly result from their effect on the nutritional condition (or body mass) of the nestlings (pathways 3 and 4, respectively), when condition in turn affects the immune response (pathway 7). Finally, the variation in phenotypic quality among the nestlings at the start of the cross-foster experiment, here given by body mass on day 1, may account for common origin and common rearing environment effects on cell-mediated immunity directly (pathway 5) or indirectly through their effect on variation in body mass on day 12 (pathways 6 and 7).

Figure 1 summarizes the possible pathways for variation in cell-mediated immunity among nestlings. In each nested ANOVA model, cross-foster group was the main nesting factor, with nest of origin (random effect) nested as a factor within the cross-foster group (random effect). The term cross-foster group accounts for common environmental effects such as season. A significant nest-of-origin effect would indicate origin-related variation in cell-mediated immunity (pathway 2 in figure 1) or eventual body mass (day 12; pathway 4 in figure 1) among nestlings. Further, to control for the effect of initial size differences between sibling groups, body mass at day 1 (fixed effect) was included in each analysis of cell-mediated immunity (pathway 5 in figure 1) and body mass on day 12 (pathway 6 in figure 1).

First, we investigated the variance in host susceptibility to hen fleas due to a common origin. To control for between-nest variation in body mass on day 12, the individual nestling's deviation from the brood mean was used as an estimate for origin-related growth. We compared the residual variation in body mass between sibling groups raised in flea-infested and ectoparasite-free nests and predicted a significant interaction

Table 1. *Nested analyses of variance showing the effect of nest of rearing and nest of origin on cell-mediated immunity and body mass of nestlings at day 12*

(Individual nestlings were assigned to their specific cross-foster group, nest of rearing and nest of origin. To investigate the effect of rearing locality and parentage, nest of rearing and nest of origin were entered as a factor whose effect was nested in that of the cross-foster group. The variance components were tested using the Satterthwaite approximation (Sokal & Rohlf 1995; Sall & Lehmann 1996). Note that the variance components are not estimated for fixed effects (i.e. body mass on day 1 and body mass on day 12). The difference in the sample size between nest of rearing and nest of origin is due to complete failure of two broods after cross-fostering.)

			d.f.			
model	source	F	numerator	denominator	p	variance component
(1) cell-mediated immunity	group	1.30	12	63.5	0.241	19.84
	nest of rearing	4.07	46	233	< 0.001	165.63
	nest of origin	2.43	48	233	< 0.001	79.76
	error	—	233	—	—	270.93
(2) body mass on day 12	group	1.33	12	56.6	0.228	0.22
	nest of rearing	11.64	46	232	< 0.001	2.50
	nest of origin	2.43	48	232	< 0.001	0.35
	body mass day 1	51.10	1	232	< 0.001	—
	error	—	232	—	—	1.17
(3) cell-mediated immunity	group	1.10	12	60.7	0.377	4.66
	nest of rearing	2.36	46	232	< 0.001	70.81
	nest of origin	2.46	48	232	< 0.001	78.12
	body mass day 12	12.98	1	232	< 0.001	—
	error	—	232	—	—	257.68

between flea treatment (factor; fixed effect) and origin if there was origin-related variation in host susceptibility. Second, we investigated the overall effect of hen flea infestation on cell-mediated immunity (pathway 1 in figure 1) and body mass on day 12 (pathway 3 in figure 1). We therefore tested flea treatment as a main effect while controlling for origin-related variation. Third, we investigated the variance in cell-mediated immunity due to a common origin and common rearing environment. Flea infestation was now incorporated in the overall nest-of-rearing effect (factor; random effect) which was then tested besides the nest-of-origin term as a nested effect within the cross-foster group (pathway 1 in figure 1). The nest-of-rearing term also accounts for rearing environment-related effects such as brood size and the presence of own (not cross-fostered) young. We further analysed the variance in body mass on day 12 and investigated to what extent origin-related or rearing environment-related variation in body mass (pathways 4 and 3 in figure 1) explained the variation in cell-mediated immunity (pathway 7 in figure 1). Body mass on day 12 was therefore tested as a covariate besides the nest-of-rearing and nest-of-origin terms.

Prior to fitting nested ANOVA models we tested whether the dependent variables wing web swelling or body mass on day 12 were normally distributed within each of the 13 cross-foster group using Shapiro–Wilk W-tests (Sall & Lehmann 1996). Significant but small ($W > 0.96$) deviations from the normal were found in one case for wing web swelling and in two cases for body mass. We Box–Cox transformed the data (Sokal & Rohlf 1995), but a significant deviation from a normal distribution was again found both in wing web swelling and body mass on day 12 in one cross-foster group. As the results and conclusions of our analyses were virtually identical using transformed and untransformed data, we present the results for the untransformed data only. All statistical tests are two-tailed.

3. RESULTS

(a) *Variation in host susceptibility to hen fleas*

The variation in residual body mass among individual nestlings was analysed in a mixed-model ANOVA, with cross-foster group (group) as the main nesting factor, nest of origin (origin) nested as a factor within the group, flea treatment (treatment) as a factor and the interaction between treatment and origin nested within the group. The interaction between flea treatment and origin was not significant ($F_{48,230} = 1.17$ and $p = 0.22$). Thus, there was no evidence that nestlings of different origin varied in relative growth between non-infested and infested broods.

(b) *Overall effect of flea infestation on cell-mediated immunity and body mass*

The mean nestling body masses on day 12 ($\pm$s.e.) were 14.56 g ($\pm$0.35 g) and 14.26 g ($\pm$0.26 g) for the uninfested and infested nests of rearing, respectively. Thus, the nestlings in flea-infested broods were on average lighter than in uninfested broods, but this difference was not significant (t-test $F_{1,57} = 0.50$ and $p = 0.48$). The mean wing web indices in the uninfested and infested nests were 70.93 ($\pm$3.17) and 72.91 ($\pm$2.39), respectively and were independent of treatment ($F_{1,57} = 0.26$ and $p = 0.61$). In a second test for the overall effect of flea infestation, we statistically controlled for the variance explained by cross-foster group and origin (mixed-model nested ANOVA). This analysis also indicated a non-significant contribution of flea infestation to the explained variance in both cell-mediated immunity ($F_{1,278} = 0.027$ and $p = 0.87$; pathway 1 in figure 1) and body mass ($F_{1,278} = 2.02$ and $p = 0.16$; pathway 3 in figure 1).

(c) *Variation in cell-mediated immunity due to a common origin*

The previous analyses showed no overall effect of flea infestation on the cell-mediated immunity or body mass of the nestlings. We therefore decided not to discriminate between uninfested and infested nests in further analyses, but to include the effect of flea infestation in the nest-of-rearing effect, which controls for other aspects of the rearing environment as well.

The cell-mediated immunity of the nestlings was significantly related to nest of rearing and nest of origin, while cross-foster group was not significant (table 1, model 1). In addition, body mass at day 1 did not contribute significantly to the explained variance ($F_{1,232} < 0.0006$ and $p = 0.98$; pathway 5 in figure 1); thus, the variance in initial size between chicks of different origin was not responsible for the origin effect or rearing environment effect on the cell-mediated immune response. The statistically significant effect of nest of rearing indicated that the differences in cell-mediated immunity between nestlings were partly due to factors related to the rearing environment. The effect of rearing environment appeared at least partly due to brood size, since we found a negative correlation between the brood size and cell-mediated immunity of nestlings when controlling for the effect of group and origin ($F_{1,278} = 7.67$ and $p = 0.006$). The significance of nest of origin (table 1, model 1) indicated that genetic variance and/or maternal effects contributed to the variation in cell-mediated immunity among the nestlings, irrespective of the actual rearing environment of a nestling. Examination of the causal components of variance indicated that nest of rearing explained *ca.* 31% of the variation in the cell-mediated immune response among nestlings, while nest of origin accounted for *ca.* 15% (table 1, model 1).

The body mass of nestlings at age 12 days was significantly related to nest of rearing and nest of origin, while cross-foster group was not significant. In addition, body mass on day 1 explained a significant part of the variance (table 1, model 2; pathways 3, 4 and 6 in figure 1, respectively). Body mass on day 12 was positively related to body mass on day 1 (regression coefficient 1.41). The incorporation of body mass on day 1 in the model reduced the variance explained by the origin by nearly half (8 versus 15%; table 1, model 2), suggesting that the initial size differences contributed significantly to the resemblance in body mass on day 12 among nestlings of the same origin. Controlling for the variation in initial body mass, nest of rearing explained a major part of the total variance in body mass among the nestlings, i.e. 59% (table 1, model 2). The strong effect of rearing environment on body mass indicates substantial variation in the conditions for nestling growth among different nesting locations, probably due to variation in the food supply. Rearing environment-related differences in nestling growth were partly explained by offspring number, as nestling body mass was negatively related to brood size (controlling for the effect of cross-foster group, origin and body mass on day 1; $F_{1,278} = 32.08$ and $p < 0.0001$). The significance of nest of origin (table 1, model 2) suggests genetic variance in body mass among nestlings.

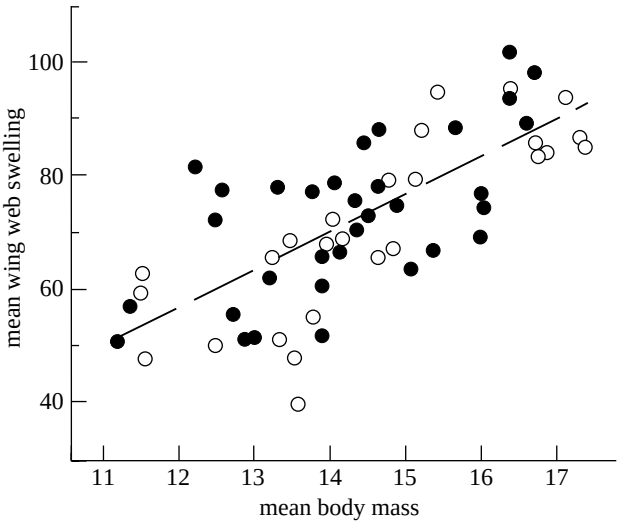

Figure 2. Relationship between mean body mass and mean cell-mediated immune response among nests of rearing. Filled circles denote nests experimentally infested with hen fleas at the start of the experiment and open circles indicate non-infested nests.

The mean wing web swelling in foster broods was positively related to the mean body mass ($F_{1,57} = 62.13$ and $p < 0.0001$; figure 2), suggesting that nestlings of relatively high body mass had a stronger cell-mediated immunity than relatively light nestlings. Given the effect of rearing environment and origin on body mass on day 12 (table 1, model 2) and given the positive relationship between the mean body mass on day 12 and the mean cell-mediated immune response in the nest of rearing (figure 2), we finally investigated whether the variation in body mass on day 12 among nestlings explained the effect of rearing environment or origin on the cell-mediated immunity (pathways 1 and 2 by pathway 7 in figure 1). We therefore added body mass on day 12 as a covariate to the basic model of table 1, model 1. Body mass on day 12 explained a significant part of the individual variation in wing web swelling, but, in addition, nest of rearing and nest of origin remained significant, while the factor group was again not significant (table 1, model 3). The cell-mediated immune response was positively related to body mass, as shown previously by the mean values per foster brood (figure 2). Interestingly, when controlling for body mass on day 12, the variance component associated with nest of rearing decreased considerably (reduction of 57%; see table 1, model 1), while the variance component estimated for nest of origin was hardly affected (table 1). This is in accordance with the strong effect of rearing environment on nestling growth (table 1, model 2 and pathway 3 in figure 1), with body mass on day 12 in turn affecting cell-mediated immunity (pathway 7 in figure 1). Individual variation in nestling body mass on day 12 thereby essentially explained the rearing environment-related variance in immunocompetence associated with brood size (contribution of brood size when replacing nest of rearing in table 1, model 3; $F_{1,277} = 0.098$ and $p = 0.75$). The nest-of-origin effect on cell-mediated immunity was essentially independent of nestling body mass.

4. DISCUSSION

(a) *Origin-related variation in susceptibility to hen fleas*

We found no evidence of origin-related variation in susceptibility to hen fleas as sibling groups showed similar relative growth under the ectoparasite-free and flea-infested conditions. However, there was also no overall effect of hen fleas on nestling body mass, suggesting circumstances of high host resistance, high host tolerance or low parasite virulence in general. The low impact of hen fleas in the present year of study might have precluded the expression of heritable variation in host susceptibility, but we have currently no data to support this hypothesis.

(b) *Effect of flea infestation on cell-mediated immunity*

The nestlings' cell-mediated immunity was not significantly affected by flea infestation. Given the demonstrated low effect of fleas on body mass, an altered cutaneous hypersensitivity response was not expected on the basis of a parasite-mediated nutritional condition effect. A comparable result was found in the barn swallow, where young in nests inoculated with additional louse flies showed a similar variation in body mass and cell-mediated immunity as nestlings in control broods (Saino *et al*. 1998). Both studies produced no evidence of an immunological trade-off, i.e. specific ectoparasite-directed immune responses lowering cell-mediated immunity or, alternatively, that ectoparasite infestation induces higher T-cell activity in general. At present more data are needed, particularly under conditions of significant parasite virulence, to draw any conclusions regarding the effect of ectoparasites on nestling immunocompetence.

(c) *Origin-related variation in cell-mediated immunity*

The variation in cell-mediated immunity among the nestlings was clearly associated with the nest of origin, suggesting heritable variation. The cutaneous hypersensitivity response to a primary challenge with PHA provides a measure of the proliferative potential of circulating T lymphocytes (Goto *et al*. 1978). Therefore, consistent variation in this response among sibling groups in great tits might indicate the existence of genetic variation in T-cell reactivity or in T-cell numbers. The influence of genetic origin on cell-mediated immunity has been demonstrated using selection lines of poultry (Cheng & Lamont (1988) and references therein) and the response to PHA has been shown to be associated with the major histocompatibility complex (Clare *et al*. 1985; Taylor *et al*. 1987). Only the partial cross-foster study by Saino *et al*. (1997*a*) has provided further evidence for origin-related variation in T-cell responsiveness under natural conditions.

It needs to be noted that we assumed that nestlings from the same nest were full-sibs. This assumption might be violated due to the presence of extra-pair young. We have no data on the relatedness of parents and their putative offspring, but other population studies in great tits have shown that, although egg dumping is virtually absent, extra-pair paternity may occur (reviewed by Verboven & Mateman 1997). Therefore, in the present study some nestlings of the same origin might have been half-sibs only, with the effect that additive genetic variance in cell-mediated immunity might have been underestimated. This renders our conclusion on the existence of genetic variation rather conservative.

T-cell immune responsiveness further showed a clear positive correlation with nestling body mass. This finding is in accordance with several studies on laboratory and domestic animals, which often show a positive correlation between nutritional condition and immunocompetence (Gershwin *et al*. 1985; Cook 1991). Cell-mediated immunity is particularly affected by nutritional condition. Captive northern bobwhite (*Colinus virginianus*) chicks showed reduced cell-mediated immunity when raised on a low-protein diet, while their humoral immune response (to sheep red blood cells) was not significantly affected (Lochmiller *et al*. 1993). In addition, there is, to our knowledge, only a single bird study which has experimentally shown the effect of diet quality on immune function under natural conditions. Saino *et al*. (1997*a*) found that food supplementation during the nestling period enhanced cell-mediated immunocompetence in nestling barn swallows.

Most of the variation in body mass on day 12 among the nestlings was explained by the differences between the rearing environments, suggesting substantial variation in food supply to individual nestlings between nests. Brood size partly accounted for the variation in nestling growth between rearing environments as we found a negative correlation between brood size and nestling body mass. This relationship might be causal, since brood size manipulation studies in birds have often shown a decrease in per capita feeding rate and nestling body mass following brood enlargement, while the opposite has been found following brood reduction (Martin 1987; Stearns 1992; Saino *et al*. 1997*a*). The variation in nestling body mass between rearing environments might also have arisen from spatial variation in food abundance, notably caterpillars (Perrins 1991; Van Noordwijk *et al*. 1995), as well as from differences between breeding pairs in parental effort. In particular, the rearing environment-related differences in conditions for nestling growth thereby explained approximately half of the variation in cell-mediated immunocompetence between different nests of rearing. The similarity in final body mass between full sibs in different rearing environments further indicated origin-related variation in nestling growth. This finding is in accordance with previous cross-fostering studies in great tits (Van Noordwijk *et al*. 1988; Gebhardt-Henrich & Van Noordwijk 1991) and may reflect heritable variation in nestling growth. However, our study gives further evidence for the potentially confounding role of initial size differences between sibling groups on heritability estimates in a cross-foster design (Van Noordwijk 1988). The initial differences in body mass at cross-fostering between sibling groups explained approximately half of the variance in eventual body mass, which had previously been assigned to an origin effect. However, pathway analysis showed that origin-related variation in initial or final nestling body mass did not account for the differences in cell-mediated immunity among sibling groups.

Origin-related variation in cell-mediated or humoral immunity, as assessed by non-specific antigens, might

indicate genetic variation in resistance against parasites. For instance, chicken lines selected for a high antibody response against sheep red blood cells were more resistant to parasites and viruses (but not bacterial infections) than those of the low-antibody production line (Gross *et al*. 1980). Consistent with the idea that improved acquired immunity may enhance fitness under natural conditions is the observation that the survival of male barn swallows was positively correlated with the sheep red blood cell antibody response in the previous breeding season (Saino *et al*. 1997*b*). Furthermore, following brood size enlargement, female collared flycatchers (*Ficedula albicollis*) had a lower antibody response to Newcastle disease virus and increased intensity of blood parasites (*Haemoproteus*) and such infections were associated with a higher mortality up to the next breeding season (Nordling *et al*. 1998). Nevertheless, as pointed out by others (Norris *et al*. 1994; Sheldon & Verhulst 1996), such data do not prove that there was a causal link between immunocompetence, host resistance to parasites and fitness components.

Summarizing, our study demonstrated origin-related variation in one important aspect of immune function, i.e. cell-mediated immunity. Forthcoming studies using various non-specific antigens to measure several aspects of immunity (innate, humoral and cell-mediated) will produce a more complete picture of total immunocompetence (Sheldon & Verhulst 1996) and potentially reveal genetic correlations between different components of the immune system (Wakelin & Apanius 1997). However, such studies are unable to produce a direct link between immune function and fitness. To establish a functional relationship between genetic variation in immune function, host tolerance and fitness, the use of specific parasite-related antigens and parasite-directed antibodies might be a more promising avenue for future studies.

Many thanks are due to G. Kralidis and H. Riedwyl for statistical advice. This study was supported by grants, nos 31–43570.95 and 31–53956.98 from the Swiss National Science Foundation to H.R.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.