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Genetic and environmental variation in immune response of collared flycatcher nestlings

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Abstract

This paper aims at partitioning genetic and environmental contribution to the phenotypic variance in nestling immune function measured with the hypersensitivity test after inoculation with phytohaemagglutinin. A cross-fostering experiment with artificial enlargement of some broods was conducted. Variation in nestling immune response was related to their common origin, which suggests heritable component of cell-mediated immunity. A common rearing environment also explained a significant part of variation. However, deterioration of rearing conditions as simulated by enlargement of brood size did not affect nestling immunocompetence, although it affected nestling body mass. Variation in body mass explained some of the variation in immune response related to rearing environment, which means that growth is more sensitive to the shifts in rearing conditions than the development of immune function. Heritable variation in immune response suggests that there should be potential for selection to operate and the micro evolutionary changes in immunity of flycatcher nestlings are possible.

Introduction

Parasites constitute an important selective force in the evolution of organisms. For example, parasites may drive evolution of host sexual reproduction (Hamilton, 1980), and may play an important role in sexual selection (Hamilton & Zuk, 1982) and the evolution of life histories (Stearns, 1992). In response to parasites hosts, have evolved numerous defence mechanisms among which the immune system of vertebrates seem to be the most effective when the host has already contracted infection. The immune system serves also as an effective scavenger of endogenous aberrations of cell functions (Roitt *et al.*, 1996). Thus, variation in immune function may constitute an important selective target, as immunity should affect individual fitness. However, an evolutionary response to selection is possible only if a considerable phenotypic variability exists between individuals in immune function that is at least partly heritable (Falconer & Mackay, 1996).

A significant genetic component has been found to be an important determinant of phenotypic variance in parasite load and immune responsiveness in controlled laboratory conditions (e.g. Taylor *et al.*, 1987; Cheng *et al.*, 1991; Kean *et al.*, 1994; Goater & Holmes, 1997). However, heritability estimates are usually higher in laboratory conditions than in wild populations (Sorci *et al.*, 1997) and such estimates may not reflect evolutionary potential. To date, genetic contribution to the phenotypic expression of traits related to parasite resistance under natural conditions has been detected in several studies (Saino *et al.*, 1997; Brinkhof *et al.*, 1999; Soler *et al.*, 2003), but found nonsignificant in others (Christe *et al.*, 2000; Tella *et al.*, 2000a). Strong evidence has been presented that host resistance may be affected by environmental factors such as food availability (Gershwin *et al.*, 1985; Lochmiller *et al.*, 1993; Saino *et al.*, 1997).

In this study, we investigate the relative importance of genetic and environmental factors in determining cell-mediated immune response of collared flycatcher

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(*Ficedula albicollis*) nestlings. We used a standard hypersensitivity test to the phytohaemaglutinin (PHA), which provides a measure of the proliferate ability of T-lymphocytes (Goto *et al.*, 1978). This test is widely used in the studies of bird immunocompetence (Brinkhof *et al.*, 1999; Christe *et al.*, 2000, Tella *et al.*, 2000a). We also analyse variation in nestling's body mass, which has been repeatedly reported to covary with nestling immune response in several bird species (e.g. Saino *et al.*, 1997; Brinkhof *et al.*, 1999). Particularly, we investigate how different environmental conditions, as simulated by brood size alterations, affect causal components of phenotypic variance in nestling immunocompetence. We performed a cross-fostering experiment and enlarged some broods by adding extra nestlings to simulate good and poor rearing conditions.

Study area and methods

The study was conducted in a population of collared flycatchers breeding in nest boxes on the island of Gotland, Sweden, in 2001 and 2002 (for details about the study area see Gustafsson, 1989). The Collared Flycatcher is a small (ca. 13 g) migratory passerine bird species breeding mainly in eastern and central Europe and wintering in southern and central Africa. It nests in natural tree cavities, but prefers nest boxes when these are provided.

In order to explore genetic and environmental contributions to the phenotypic variance in nestling immunocompetence and body size two separate experiments with reciprocal cross-fostering of nestlings were performed. In both experiments, nestlings were swapped between triplets of nests matched in terms of equal hatching date and brood size at the second day after hatching. All nestlings were marked individually by nail clipping, weighed with a 5 g Pesola (accuracy 0.01 g) and transferred in a warmed box to a paired nest. All manipulation at the nest and the transfer took up to 30 min for a triplet of nests. In such a way, we created broods with approximately equal number of nestlings from three different families. In the first experiment nestlings were only cross-fostered without altering brood size and in the second experiment broods were cross-fostered and additionally some randomly chosen broods within a triplet (in some triplets it was one brood and in other two broods) received two extra nestlings while some broods within a triplet were left un-manipulated. These extra nestlings were of the same age and came from a donor nest not included in triplets. One donor nest usually provided extra nestlings to more than one nest involved in broods size manipulation. Brood size manipulation aimed at introducing additional environmental variance in a focal trait by creating, in some nests good, and in the other nests poor rearing conditions, which allowed testing significance of genetic component under experimentally controlled environment. In total, nine triplets of unmanipulated broods (Experiment 1) and 11 triplets with enlarged broods (Experiment 2) were created in 2001 and seven unmanipulated triplets and eight triplets with enlarged broods in 2002. Altogether there were 240 nestlings in the unmanipulated triplets and 285 nestlings in triplets with enlarged broods.

The cellular immune response of nestlings was stimulated by the injection of phytohaemaglutinin 11 days after hatching. PHA provokes T-cell-mediated immunity and applicability of this procedure to assess nestling immunocompetence has been confirmed in a number of studies (e.g. Lochmiller *et al.*, 1993; Brinkhof *et al.*, 1999). Nestlings were injected with 0.2 mg of PHA dissolved in 0.04 mL PBS in the right wing web and with 0.04 mL PBS in the left wing. The thickness of the wing web was measured only by one person (JS) with a pressure sensitive spessimeter (Mitutoyo, SM-12) prior to injection and 24 h after injection. Each measurement was taken three times (repeatability: prior to injection $r = 0.92$, $F_{498,998} = 35.71$, $P < 0.001$; post-injection: $r = 0.69$, $F_{477,956} = 7.68$, $P < 0.001$; Lessels & Boag, 1987). Immune response was calculated as a relative change in the mean thickness prior to and 24 h after the injection with PHA in relation to a change of the other wing injected with PBS. Nestlings were weighed with a pesola spring balance (accuracy 0.1 g) prior PHA inoculation and the day after. In the analyses of body mass we used data from the first measurement.

In order to partition genetic and environmental contribution to the observed variance in immune response and nestling body size related to common origin and common rearing, mixed procedure in SAS (SAS Inst. Inc., Cary, NC, 2000) was employed. Nest of rearing and nest of origin, both nested in nests triplet (later referred to as blocks) and year, were random factors. Differences between years and blocks (nested in year and defined as random factor) were additionally accounted for (Merilä, 1996). In such a model, a significant effect of original nest would indicate the genetic component, which in fact estimates variation attributable to the half of the additive genetic variance, but also includes a quarter of the dominance variance and potential parental effects. A significant effect of the rearing nest would indicate that environmental effects contribute to the development of nestling immunocompetence. The data obtained from the second experiment in which some broods in the triplets experienced brood enlargement were analysed with a similar model in which additional fixed effect of experiment accounted for environmental variance due to brood size manipulation. Since nestlings' immune response may depend on body mass, we performed further analyses of determinants of immune function with body mass introduced as a covariate.

In this paper, we are interested in the relative contribution of variance related to nestling origin and rearing after factoring out contribution of fixed factors. Therefore, in all analyses we calculated per cent of variance

explained only by random variables and residual variance. The per cent of variance was calculated from estimates of variance components obtained from SAS MIXED procedure with Restricted Maximum Likelihood method of estimation and Satterthwaite methods for calculation of degrees of freedom. Note that our estimates of variance do not include variance explained by fixed factors. The significance of estimates of variance components relayed on *Z*-statistics obtained with covtest option in the mixed procedure and significance of fixed factors was based on *F*-statistics obtained from Type-3 tests.

Results

First, we performed an analysis restricted to broods that were not involved in the brood size experiment (see Table 1). Both the nest of origin and rearing significantly affected nestling cell-mediated immune response, accounting for 12.6 and 17.1% of the variance respectively. Differences between years were significant. This means that both genetic (nest of origin) and environmental factors (nest of rearing) were important determinants of phenotypic variance in immunocompetence. The interaction between the nest of origin and the nest of rearing was not significant. Similar results were obtained in the analyses of determinants of phenotypic variance in nestling body mass: both the nest of origin and the nest of rearing explained a significant part of variation. The nest of rearing explained 38.6% of variation and nest of origin 20.9%, which is higher than for nestling immune response. As immune response of nestlings may depend on body mass we performed an additional analysis in which nestling body mass prior to PHA inoculation was introduced as a covariate in the model explaining phenotypic variance in immune response. Body mass indeed affected immune response, and in such a model the per cent of variance associated with the nest of rearing was considerably reduced, whereas the variance component explained by nest of origin increased. This is in accordance with the strong effect of rearing environment on nestling body mass. The body mass essentially explained the variance in nestlings' immune response associated with differences in rearing environment.

The immune response of nestlings was not significantly affected by brood size manipulation; however, it was affected by the nest of origin (see Table 2). Nest of origin accounted for 18.2% of variation. This means that the environmental variation as simulated by different rearing conditions in unaltered and enlarged broods, does not contribute significantly to the phenotypic variance in nestlings' immunocompetence. The effect of nest of rearing was also non-significant. Immune response differed between years. The interactions between experimental group and nest of origin and between nest of rearing and nest of origin were not significant which means that families responded similarly to different experimental conditions.

In contrast to immune response, body mass of nestling was negatively affected by experimental brood enlargement (Table 2). It was also significantly related to the nest of origin and nest of rearing, implying significant genetic and environmental components. Body mass of nestlings did not differ between years. Families responded similarly to different experimental conditions, as

Table 1 Environmental (nest of rearing) and genetic (nest of origin) contribution to the phenotypic variance in nestling cell-mediated immunocompetence. Block denotes cross-foster group of nests between which nestlings were transferred. Var is percentage of total variance explained by random factors calculated from variance component estimates from SAS MIXED procedure. Significance of estimate of variance components was tested with *Z*-statistics, and significance of fixed factors by F statistics obtained from Type-3 analyses. (a) Model without nestling body mass as explanatory variable and (b) with nestling body mass as an explanatory variable.

	Immune response				Body mass			
	Estimate ± SE	F_{df}/Z	*P*	Var (%)	Estimate ± SE	F_{df}/Z	*P*	Var (%)
(A)								
Year		$4.70_{1,12.2}$	0.05			$0.21_{1,14.6}$	0.65	
Origin (Block × Year)	50.52 ± 27.29	1.85	0.032	12.6	0.71 ± 0.28	2.55	0.006	20.9
Rearing (Block × Year)	68.69 ± 33.37	2.06	0.02	17.1	1.30 ± 0.41	3.15	0.001	38.6
Rearing × Origin (Block × Year)	0			0	0.17 ± 0.15	1.17	0.12	5.27
Block (Year)	2.33 ± 30.07	0.08	0.47	0.58	0.38 ± 10.46	0.83	0.20	11.3
Error	280.39 ± 30.80	9.10	<0.0001	69.8	0.81 ± 0.11	7.63	<0.0001	23.9
(B)								
Year		$6.76_{1,45.2}$	0.013					
Body mass		$9.50_{1,45.2}$	0.002					
Origin (Block × Year)	61.85 ± 28.76	2.15	0.016	16.3				
Rearing (Block × Year)	36.91 ± 24.73	1.49	0.068	9.70				
Rearing × Origin (Block × Year)	0			0				
Block (Year)	0			0				
Error	281.45 ± 31.09	9.05	<0.0001	75.2				

Table 2 Environmental (nest of rearing) and genetic (nest of origin) contributions to the phenotypic variance in nestling cell-mediated immunocompetence among broods experiencing experimental brood size manipulation. Block denotes cross-foster group of nests between which nestlings were transferred. Var is percentage of total variance explained by random factors calculated from variance component estimates from SAS MIXED procedure. Significance of estimate of variance components was tested by *Z*-statistics, and significance of fixed factors by *F*-statistics obtained from Type-3 analyses. (a) Model without nestling body mass as explanatory variable and (b) with nestling body mass as an explanatory variable.

	Immune response				Body mass			
	Estimate ± SE	F_{df}/Z	*P*	Var (%)	Estimate ± SE	F_{df}/Z	*P*	Var (%)
(A)								
Year		$10.92_{1,47.4}$	0.002			$0.29_{1,17.1}$	0.59	
Experiment		$0.03_{1,28.2}$	0.87			$8.18_{1,15.9}$	0.01	
Year × Experiment		$0.01_{1,28.2}$	0.95			$4.11_{1,15.9}$	0.06	
Origin(Year × Block)	77.67 ± 29.09	2.67	0.004	18.2	0.25 ± 0.11	2.27	0.011	8.9
Rearing (Block × Year × Experiment)	24.34 ± 29.36	0.83	0.20	5.7	0.70 ± 0.31	2.23	0.013	24.3
Rearing × Origin(Block × Year)	0			0	0			0
Experiment × Origin(Block × year)	0			0	0			0
Block (Year)	0			0	0.08 ± 0.44	0.2	0.42	2.9
Block (Year) × Experiment	26.54 ± 32.17	0.83	0.20	6.21	0.82 ± 0.59	1.39	0.08	28.2
Error	298.33 ± 29.98	9.95	<0.0001	69.9	1.43 ± 0.11	9.91	<0.0001	35.7
(B)								
Year		$11.39_{1,46.4}$	0.002					
Experiment		$0.11_{1,29.9}$	0.74					
Year × Experiment		$0.12_{1,29.5}$	0.73					
Body mass		$4.44_{1,204}$	0.036					
Origin(Year × Block)	74.83 ± 28.01	2.67	0.0038	17.9				
Rearing (Block × Year × Experiment)	27.09 ± 29.07	0.93	0.18	6.5				
Rearing × Origin(Block × Year)	0			0				
Experiment × Origin(Block × Year)	0			0				
Block (Year)	0			0				
Block (Year) × Experiment	16.82 ± 30.04	0.56	0.29	4.0				
Error	298.11 ± 29.94	9.96	<0.0001	71.51				

indicated by nonsignificant interactions between experimental treatment and nest of origin and between nest of rearing and nest of origin, thus genotype–environmental interactions seemed not to be present. Body mass, introduced as a covariate in the model exploring variation in nestling immune response, significantly affected immune response. In such analysis, the effect of the nest of origin was still significant and explains similar amount of variation, while nest of rearing was not significant.

Discussion

Our data suggest that the cell-mediated immune response and body mass of collared flycatcher nestlings have a significant genetic component. Environmental factors appeared to be significant determinants of phenotypic variance in these traits, but body mass was relatively more affected by rearing environment than the immune response: in contrast to body size, immune response was not affected by brood size manipulation, and the effect of nest of rearing was small and nonsignificant among broods involved in brood size experiment. Body mass explains a significant part of variation in immune response and considerably reduces the environmental component of variance in immune response.

These results clearly indicate that the development of immune function is less sensitive to variation in rearing environment than body growth. One could speculate that this may reflect different priorities of investments to the development of immune function and body size. Limited resources should be preferentially invested to the development of traits, which are more closely related to fitness, and the immune function might indeed be more critical for survival of the nestlings than body size (Cichoń & Dubiec, 2005). Developmental priority of immune functions should be expected if the risk of contracting infection is high and relative costs of developing immune function are low. The costs of development of immunity are not known, but our unpublished data suggest that the risk of contracting infection in the studied population of collared flycatcher might be substantial (prevalence of some haematozoan parasites could be up to 30–40%). If prevalence of parasites and costs of development of immunity differ between populations or species, the environmental effects and the outcomes of brood size manipulations may vary between populations.

Significant genetic and environmental components of variation in body mass have been repeatedly reported in earlier studies (e.g. Garnett, 1981; Gebhardt-Henrich & van Nordwijk, 1991; Merilä, 1996; Brinkhof *et al.*, 1999). There is also growing evidence of a significant genetic contribution to the phenotypic variance in immune response in birds from wild populations (Saino *et al.*, 1997; Brinkhof *et al.*, 1999; Christe *et al.*, 2000; Tella *et al.*, 2000b). In poultry, Cheng & Lamont (1988) demonstrated a significant genetic change in cell-mediated immunity in response to artificial selection. A significant directional or stabilizing selection on humoral immune response to two different antigens (Råberg & Stjernman, 2003) and a directional selection on cell-mediated immunity (Cichoń & Dubiec, 2005) have been recently reported in the field studies on blue tits. However, the data from the wild populations suggest that environmental factors are the most important determinants of the immunity of nestlings (Saino *et al.*, 1997; Christe *et al.*, 2000; Tella *et al.*, 2000a). For example, artificial food provisioning with food rich in proteins usually has positive effects on nestling immunocompetence (e.g. Saino *et al.*, 1997). Nestling cell-mediated immune response has been found to be negatively related to brood size and it was significantly lower among nestlings from experimentally enlarged broods than those from unmanipulated and reduced broods (Saino *et al.*, 1997; Hõrak *et al.*, 1999; Saino *et al.*, 2003; but see Bonneaud *et al.*, 2003).

One needs to acknowledge the existence of some potentially confounding factors that could affect the estimations of genetic contribution to phenotypic variance. (i) The effect of additive genetic variance reported in this study is probably underestimated due to extra pair paternity, which may result in lowered father-offspring resemblance if some nestlings from the same nest of origin might have been half sibs. In our population of collared flycatchers, about 15% of nestlings originate from extra pair copulations (Ellegren *et al.*, 1996). (ii) Genetic variance, as estimated from the effect of nest of origin, may also be overestimated in our study as it potentially includes a component of maternal or early common-environment effects. Chicks were swapped 2 days after hatching, so differences in egg provisioning and early parental care were included in our estimates of genetic variances.

A significant genetic component is a prerequisite for selection to operate and the magnitude of genetic component reflects the scope for micro evolutionary response to selection of the phenotypic trait (Charlesworth, 1994; Houle, 1992). Immune function may play a critical role for survival if an individual contracts an infection but its function may be costly to maintain if the risk of infection is low. Thus, selection may operate in opposite directions depending on the risk of infection. The significant genetic variation may thus indicate that selection can indeed operate and more importantly that there is a possibility for an evolutionary response in the level of immune function. It is important to note here that we did not find any indications of genotype-environment interactions. Such interactions would imply existence of potential genetic constraints for adaptation in unpredictable environments, as specific genotypes might be favoured under different environmental conditions. Thus, if immunocompetence, as measured in this study, reflects the potential of individuals to fight parasites, our results corroborate the basic assumption of the hypotheses of the potential role of parasites in driving evolution of sexual reproduction, sexual selection and life histories. However, to understand the role of parasites in evolution of various traits future research need to concentrate on studying genetic variation in response to specific parasite antigens encountered by individuals during their life.

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