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Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers

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The response to intradermally injected phytohaemagglutinin (PHA-response) is a commonly used quantification of avian immunocompetence (the ability to resist pathogens). Parasite-mediated sexual selection requires heritable immunocompetence, but evidence for heritability of PHA-response in birds largely stems from full-sib comparisons. Using an animal model approach, we quantified the narrow-sense heritability of PHA-response in 1626 collared flycatcher (*Ficedula albicollis*) nestlings from 332 families, most of which were cross-fostered. Nestling PHA-response was not significantly heritable ($h^2=0.06\pm0.10$), but was subject to non-heritable nest-of-origin effects (10% of variation). Our findings illustrate that full-sib comparisons of immunological measures may lead to an inflated estimate of heritability and also reveal a limited role of nestling PHA-response for sexual selection in this population.

Keywords: heritability; animal model; immunity; evolutionary quantitative genetics; wild population

1. INTRODUCTION

Immune function is receiving increasing interest from evolutionary and behavioural ecologists (Norris & Evans 2000). This interest largely stems from the hypothesis of parasite-mediated sexual selection (PMSS), proposed by Hamilton & Zuk (1982). PMSS states that a male's sexual ornaments signal his heritable immunocompetence (resistance to pathogens). Females, by mating with highly ornamented males, therefore gain indirect benefits in terms of increased immunocompetence in their offspring.

The hypersensitivity response to phytohaemagglutinin (PHA-response) is the most widely used measure of immunocompetence in avian ecological studies, often in the context of PMSS (e.g. Johnsen *et al.* 2000; Møller & Petrie 2002; Saino *et al.* 2002; Garamszegi *et al.* 2003; Saks *et al.* 2003; Parn *et al.* 2005). PHA is injected intradermally, where it induces inflammation and swelling involving both innate and acquired cell-mediated elements of immunity (Martin *et al.* 2006). Laboratory studies on poultry show that PHA-response is heritable (Cheng & Lamont 1988; Sundaresan *et al.* 2005). In wild populations, estimates of heritability of PHA-response are largely based on cross-fostering studies (table 1). Such full-sib comparisons cannot separate additive genetic effects from dominance variance and early environmental (maternal) effects (Lynch & Walsh 1998), and therefore may present an inflated estimate of heritability of PHA-response.

Here, we use an animal model approach (e.g. Kruuk 2004) to estimate the causal components of variance in nestling PHA-response in collared flycatchers (*Ficedula albicollis*). Using 5 years of data on cross-fostered nestlings in a pedigreed population allows us to separate additive genetic effects from the effects of common nest environment. Our study is, to our knowledge, the first to provide an estimate of narrow-sense heritability of nestling PHA-response in a wild population.

2. MATERIAL AND METHODS

(a) *Field procedures*

The study was conducted in the southern part of Gotland (57°10′ N, 18°20′ E) in a nest-box population. Regular checking of the nest boxes allowed the establishment of laying dates, clutch sizes and hatching dates of broods. Each year, practically all nestlings and breeding adults were caught and ringed. Data on nestling PHA-response were collected during the years 2001–2002 and 2004–2006, following a protocol (Smits *et al.* 1999) where 11 days old nestlings were injected with 0.04 ml of PHA (Sigma code L8754) solution in saline ($5\ \mathrm{mg\ ml^{-1}}$) in the right wing web. The thickness of the wing web was measured to the nearest 0.01 mm with a spessimeter (Mitutoyo 700-117SU) prior to injection (two or three times) and 24 h (±1 h) after injection (three times). The immune response was calculated as mean post-injection thickness minus mean pre-injection thickness. Each year, all measurements were conducted by only one person (three measurers during 5 years). We measured body mass (with a spring balance to the nearest 0.1 g) and tarsus length (with a digital calliper to the nearest 0.1 mm) of 12 days old nestlings.

In 2004, no cross-fostering was conducted, but in other years nestlings were swapped between nests with matching hatching dates on the second day after hatching. Details of the cross-fostering design differed, but always led to nestlings originating from one family being reared in several nests. PHA-response and morphological traits were measured for 1626 nestlings from 332 broods.

(b) *Statistical analysis*

An animal model uses all available pedigree links to estimate the additive genetic variance (Kruuk 2004). We included additive genetic, nest-of-origin, nest-of-rearing and year as random effects. In order to assess whether our dataset contains enough information to reliably estimate additive genetic effects, we compared variance components for body mass and tarsus length in our dataset with results of previous work in this population (Kruuk *et al.* 2001; Merilä *et al.* 2001). Narrow-sense heritability is the ratio of additive genetic variance over phenotypic variance. We included the mean temperature of the day of injection (see Garvin *et al.* 2006) as the fixed effect on PHA-response. In 2001–2002, a brood size experiment was conducted (Cichoń *et al.* 2006), but this manipulation did not affect nestlings' PHA-response; therefore, we did not include brood size manipulation effect in our statistical models. The effect of misassigned paternities due to extra-pair fertilizations (15%; Sheldon & Ellegren 1999) on the estimation of heritability through animal model is probably negligible (Charmantier & Reale 2005). Significance of random effects was assessed with a likelihood ratio test. Analyses were conducted using ASReml v. 2.00 (VSN International).

3. RESULTS

Together, additive genetic and nest-of-origin effects explained 16.4% of variance in nestling PHA-response, but the narrow-sense heritability of nestling PHA-response was low and non-significant (0.059±0.099), whereas nest-of-origin effects were marginally significant (table 2). Additive genetic effects accounted for a

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Table 1. Studies quantifying heritability (h^2) of nestling PHA-response in avian wild populations. (In full-sib analyses, p is the significance of the nest-of-origin effect, and in parent–offspring regression the significance of the slope.)

study	species	h^2	method	p	remarks
Ardia & Rice (2006)	*Tachycineta bicolor*	0	full-sib		three different populations
		0	full-sib		
		0.42	full-sib	<0.05	
Ardia (2005)	*Sturnus vulgaris*	0.80	full-sib	<0.01	
Brinkhof *et al.* (1999)	*Parus major*	0.30	full-sib	<0.001	
Christe *et al.* (2000)	*Delichon urbica*	0.01	full-sib	0.59	
Cichoń *et al.* (2006)	*Ficedula albicollis*	0.25	full-sib	0.032	
		0.36	full-sib	0.004	brood size manipulation
Cucco *et al.* (2006)	*Perdix perdix*	0.05	mother–offspring	0.82	measured in different life stages
		0.27	father–offspring	0.20	measured in different life stages
De Neve *et al.* (2004)	*Pica pica*	0.19	full-sib	>0.05	food supplemented nestlings
		0	full-sib		control nestlings
Kilpimaa *et al.* (2005)	*Parus major*	0.07	full-sib	0.40	
Saino *et al.* (1997)	*Hirundo rustica*	no data	full-sib	0.004	
Soler *et al.* (2003)	*Ficedula hypoleuca*	0.17	full-sib	0.23	
Tella *et al.* (2000)	*Falco sparverius*	0.24	full-sib	0.048	

Table 2. Causal components of variance (V) in nestling PHA-response, body mass and tarsus length derived from animal model. (Significance of components was assessed with a likelihood ratio test, calculated as twice the difference in log likelihood (ΔlogLik) tested as a χ^2 distribution with one degree of freedom. The proportion of variance (%V) explained by additive genetic effects is the heritability h^2. For comparison, we present heritabilities of morphological traits based on a large 18-year dataset.)

						h^2 ± s.e. (in %, large dataset)	
trait	source	V ± s.e.	%V ± s.e.	ΔlogLik	p	non-cross-fostered	cross-fostered
PHA-response	additive genetic	0.0030 ± 0.0050	5.89 ± 9.92	0.17	0.560		
	nest-of-origin	0.0053 ± 0.0027	10.36 ± 5.63	1.90	0.051		
	nest-of-rearing	0.0077 ± 0.0014	15.14 ± 3.81	36.90	<0.0001		
	year	0.0134 ± 0.0098	26.26 ± 14.18	54.77	<0.0001		
	residual	0.0216 ± 0.0028	42.36 ± 9.80				
body mass	additive genetic	0.59 ± 0.12	19.47 ± 4.23	2.79	0.018	29.93 ± 2.26^{a}	21.92 ± 2.90^{a}
	nest-of-origin	0	0	0.00	1.000		
	nest-of-rearing	1.55 ± 0.16	50.95 ± 5.02	249.84	<0.0001		
	year	0.31 ± 0.24	10.23 ± 7.20	13.33	<0.0001		
	residual	0.59 ± 0.08	19.35 ± 3.18				
tarsus length	additive genetic	0.235 ± 0.038	36.98 ± 6.06	6.19	0.0004	35.26 ± 2.12^{b}	28.03 ± 4.45^{b}
	nest-of-origin	0	0	0.00	1.000		
	nest-of-rearing	0.271 ± 0.031	42.67 ± 4.24	145.61	<0.0001		
	year	0.047 ± 0.038	7.46 ± 5.59	9.51	<0.0001		
	residual	0.082 ± 0.022	12.90 ± 0.03				

[a] Tarsus-corrected body mass (Merilä *et al.* 2001).

[b] Kruuk *et al.* (2001).

significant part of variation in body mass ($h^2 = 0.195 \pm 0.042$) and tarsus length ($h^2 = 0.370 \pm 0.061$) in our data, and these estimates were comparable to previous estimates (table 2). Year explained the highest proportion of environmental variance in nestling PHA-response, whereas environmental variance in morphological traits was mainly attributable to nest-of-rearing effects (table 2). PHA-response was positively correlated with air temperature on the day of injection ($F_{1,1620} = 9.14$, $p = 0.025$).

4. DISCUSSION

We did not find evidence for heritability of nestling response to PHA in the collared flycatcher population. Our estimates of heritability for morphological traits based on the same dataset are in agreement with those derived from a larger dataset from this population (table 2), indicating that our dataset of cross-fostered nestlings has the power to detect additive genetic effects. Parents have a clear influence on PHA-response through rearing effects, although relatively small compared with how rearing affects morphological traits. Nestling PHA-response in this species seems to be highly sensitive to annual effects and external environmental conditions like temperature (cf. Lifjeld *et al.* 2002; Garvin *et al.* 2006).

Our literature review (table 1) shows that full-sib comparisons suggest heritable nestling PHA-response in six out of 10 species. In full-sib analysis, the variance component related to nest-of-origin estimates half of additive genetic variation, but also includes non-additive genetic effects (dominance variance) and early environmental effects (Lynch & Walsh 1998), i.e. pre-hatching maternal effects and any post-hatching effects occurring prior to cross-fostering. Full-sib analysis based on the data from the first two years of this study (Cichoń *et al.* 2006) showed significant nest-of-origin effect (12.6%), which indicates heritability of 25.2% when interpreted as additive genetic variation. The animal model decomposes this nest-of-origin effect into its heritable and non-heritable components. We found that true heritable effects are in fact small (5.9%) and insignificant, and are accompanied by stronger (10.4%) non-heritable nest-of-origin effects. Our results thus clearly exemplify the importance of distinguishing between nest-of-origin and additive genetic effects.

Recent studies suggest that our finding of strong non-heritable origin effects may be general. PHA-response is related to heterozygosity in song sparrows (*Melospiza melodia*; Reid *et al.* 2005) and house finches (*Carpodacus mexicanus*; Hawley *et al.* 2005). Extra-pair nestlings were shown to mount higher responses to PHA than within-pair young (both their maternal and paternal half-sibs) in bluethroats (*Luscinia svecica*; Johnsen *et al.* 2000) and common yellowthroats (*Geothlypis trichas*; Garvin *et al.* 2006), which indicates the role of genetic compatibility (or, alternatively, differential maternal investment in offspring sired by a more attractive male; Gil *et al.* 1999) rather than additive genetic effects. Furthermore, pre-hatching maternal effects are a well-established phenomenon in birds (e.g. Blount *et al.* 2002; Groothuis *et al.* 2005), and maternal influence on some aspects of immune function can be particularly strong (e.g. Grindstaff *et al.* 2003). We clearly need additional studies to estimate the narrow-sense heritability of nestling PHA-response in order to establish the usefulness of this assay for quantifying immunocompetence in evolutionary studies.

The study complies with the animal experimentation laws of Sweden.

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