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Mhc polymorphisms fail to explain the heritability of phytohaemagglutinin-induced skin swelling in a wild passerine

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Genetic estimates of the variability of immune responses are rarely examined in natural populations because of confounding environmental effects. As a result, and because of the difficulty of pinpointing the genetic determinants of immunity, no study has to our knowledge examined the contribution of specific genes to the heritability of an immune response in wild populations. We cross-fostered nestling house sparrows to disrupt the association between genetic and environmental effects and determine the heritability of the response to a classic immunological test, the phytohaemagglutinin (PHA)-induced skin swelling. We detected significant heritability estimates of the response to PHA, of body mass and tarsus length when nestlings were 5 and 10 days old. Variation at *Mhc* genes, however, did not explain a significant portion of the genetic variation of nestling swelling to PHA. Our results suggest that while PHA-induced swelling is influenced by the nest of origin, the importance of additive genetic variation relative to non-additive genetic variation and the genetic factors that influence the former in wild populations still need to be identified for this trait.

1. INTRODUCTION

Genetic contributions to the phenotypic variability of parasite resistance and immune response have mainly been demonstrated in domestic or laboratory species (Lillehoj *et al.* 2007). The genetic variation underlying those traits is however harder to detect in natural

populations (Kilpimaa *et al.* 2005), in part because of environmental variance (Sorci *et al.* 1997), but also because fitness traits have lower heritabilities than non-fitness traits (Mousseau & Roff 1987).

The phytohaemagglutinin (PHA)-induced skin swelling test is classically used *in vivo* in immunoecological studies and provokes the infiltration and/or proliferation of several types of immune cells (Martin *et al.* 2006). When added to cultures of T lymphocytes, PHA can bind T-cell receptors and initiate a complex signalling cascade (Kuno *et al.* 1986; Chilson & Kellychilson 1989), resulting in their proliferation and differentiation independently of their specificity or clonal origin (Licastro *et al.* 1993). Importantly, chicken lines have been shown to differ in swelling intensity in reaction to PHA, demonstrating significant genetic variation for this response (Sundaresan *et al.* 2005).

Since major histocompatibility complex (MHC) molecules are not only important in foreign antigen detection but also serve key roles in the activation and proliferation of T lymphocytes (Gur *et al.* 1999), several studies have investigated their role in the response to PHA (for e.g. Rubini *et al.* 1992; Kimball *et al.* 2002). In fact, *Mhc* genes have previously been found to be associated with the phenotypic variability of this response in chickens and in a wild passerine (Taylor *et al.* 1987; Bonneaud *et al.* 2005). Here, we decomposed the phenotypic variability of the response to PHA in its genetic and environmental components by cross-fostering nestling house sparrows (*Passer domesticus*) between synchronous broods. We then investigated the sources of variation of this response and provide, to our knowledge, the first examination of the implication of *Mhc* class I genes in the heritability estimates of this trait in a natural population.

2. MATERIAL AND METHODS

(a) *Field and laboratory procedures*

This study was conducted during spring 2003 at the Center d'Etude Biologique de Chizé (France) on a nest-box population of house sparrows. We analysed phenotypic data for 182 chicks across 61 broods; genotypic and phenotypic data were available for 150 chicks across 55 broods. For a total of 42 broods, two chicks were randomly cross-fostered between synchronous broods at two days of age ($n = 84$ chicks, 56 surviving to measuring). To maximize statistical power, all 61 broods were used in analyses; results were qualitatively similar when only the 42 experimental broods were included. Body mass (± 0.1 g) and tarsus length (± 0.01 mm) were measured on 5 and 10 day old chicks. PHA was intradermally injected at day 10 (0.025 mg in 0.04 ml phosphate buffered saline; Sigma L8754) and measured after 24 h as described in Bonneaud *et al.* (2005). Blood was sampled from the brachial vein of 10 days old chicks (approx. 50 µl) and of their parents (approx. 150 µl), stored in buffer/EDTA 2 mM and kept frozen at $-20°$C for subsequent DNA extraction.

We evaluated the allelic diversity of all chicks at the most variable *Mhc* class I gene family using the PCR-based denaturing gradient gel electrophoresis method (Bonneaud *et al.* 2004). Adults and chicks were genotyped using seven microsatellite markers as described in Bonneaud *et al.* (2006). We compared the microsatellite genotypes of both parents and chicks to verify paternity and identify the chicks sired from extra-pair mating. Since the microsatellite loci were highly polymorphic, we set the detection probability of extra-pair young at 0.99. Extra-pair young were accounted for and maintained in all analyses.

(b) *Statistical analyses*

Variance components for each trait were estimated in MENDEL (Lange *et al.* 2001) using the Polygenic and quantitative trait loci option, which was also designed for classical heritability studies. This model treats the data as multivariate normal. In principle, the phenotypic variance could be partitioned into independent

Table 1. Variance components for nestling body mass, tarsus length and PHA-induced skin swelling. (V_A is conflated by non-additive effects (dominance and maternal/early rearing) so that h^2 is more a reflection of broad sense than narrow sense heritability. V_E is the variance components due to environmental effects and error variance and V_H, the variance components due to a nest of rearing effect.)

	V_A		V_H		V_E		h^2	
trait	estimate	s.e.	estimate	s.e.	estimate	s.e.	estimate	s.e.
mass at day 5	5.51	1.51	0.10	0.16	0.80	1.00	0.86	0.13
mass at day 10	5.35	2.03	3.21	1.04	0.88	1.26	0.57	0.19
tarsus length at day 5	1.32	0.38	0.03	0.05	0.28	0.25	0.81	0.22
tarsus length at day 10	0.98	0.27	0.48	0.18	0.09	0.16	0.63	0.11
PHA-induced skin swelling	1771.5	1088.3	344.0	467.5	1712.3	711.7	0.46	0.19

Table 2. Contribution of the total number of *Mhc* alleles detected in house sparrow chicks, as well as the presence of the seven most common alleles (absence of the allele as the reference state), to the estimates of their swelling response to PHA. (We indicate the standard error (s.e.) of the estimate of the effect of each *Mhc* variable on the response to PHA, the coefficient of variation (CV), the heritability of the response to PHA calculated for each model, as well as the per cent heritability explained by each *Mhc* variable. We also indicate the population frequency of each *Mhc* allele. *p*-values were calculated for a standard normal probability curve.)

	allele frequency	estimate	s.e.	CV	*p*-value	residual h^2 (s.e.)	% heritability explained
number of *Mhc* alleles		−2.22	2.84	1.28	0.22	0.46 (0.15)	0.43
Mhc allele *a158*	0.50	−8.04	10.44	1.40	0.22	0.42 (0.16)	9.72
Mhc allele *a172*	0.28	−14.89	10.54	0.71	0.08	0.43 (0.16)	6.26
Mhc allele *a161*	0.23	−7.47	12.46	1.67	0.27	0.46 (0.15)	1.30
Mhc allele *a151*	0.22	0.88	12.49	14.27	0.47	0.44 (0.17)	5.18
Mhc allele *a165*	0.21	11.33	12.32	1.09	0.18	0.48 (0.15)	0.00
Mhc allele *a163*	0.20	−16.22	12.70	0.78	0.10	0.41 (0.16)	12.3
Mhc allele *a123*	0.16	17.09	10.67	0.63	0.06	0.39 (0.16)	16.5

environmental (V_E), shared nest of rearing environmental/household (V_H), additive genetic (V_A), dominance genetic (V_D) and maternal/early environmental components (V_M) (see the electronic supplementary material, methods). The design of this study precludes separating V_A, V_D and V_M, so that only V_A is included in the model. Heritability was calculated as $h^2 = V_A/V_P$, where V_P is the observed phenotypic variance and the sum of all the components.

The statistical significance of heritability estimates were determined by calculating the difference of the max log-likelihoods obtained from the models with and without the additive effect (see the electronic supplementary material, methods; Self & Liang 1987). The statistical significance of the rearing environment (V_H) was estimated in the same way. We investigated the contribution of: (i) the total number of *Mhc* alleles in chicks, and (ii) the presence or absence of the seven most common *Mhc* alleles in the population (Bonneaud *et al.* 2006), to the variability of the response to PHA using a measured genotype approach that treats the genotype as a fixed effect in the mean part of the variance component model (Boerwinkle *et al.* 1986; Lange *et al.* 2005) and by incorporating each factor separately into the model. Statistical significance was determined using a standard *Z*-test.

3. RESULTS

Body mass, tarsus length and response to PHA all displayed significant nest of origin effects ($p < 0.001$ for measures of body mass and tarsus length and $p = 0.029$ for the response to PHA; table 1), suggesting that they were significantly heritable. As expected, nest of rearing effects increased with chick age for both body mass and tarsus length. There was a significant effect of nest of rearing for body mass and tarsus length only ($p < 0.001$), not for the response to PHA

($p = 0.21$). As a result, both shared origin and the rearing environment affected chick development and condition, although PHA-induced swelling was mostly dominated by nest of origin effects.

Mhc genes, however, assessed either as the total number of alleles, or the presence or absence of the seven most common alleles, did not explain a significance portion of the phenotypic variability of the response to PHA (table 2).

4. DISCUSSION

Nestling skin swelling displayed a significant nest of origin effect and was the only nestling parameter not to be affected by the rearing environment. Significant genetic variation in the response to PHA has rarely been demonstrated in wild bird populations (Cichón *et al.* 2006). Since heritability estimates of immunocompetence have, however, been shown to exhibit lower values in poor-quality compared with high-quality environments (De Neve *et al.* 2004), it is possible that rearing conditions were, in our study, sufficiently homogeneous between nests to allow for the expression of a nest of origin effect on this trait.

We found that the heritability of nestling response to PHA was not attributable to variation in *Mhc* genes. One possibility is that the lack of association results from the timing of measure of the response. Indeed,

Taylor *et al.* (1987) found that *Mhc* genotypes were significantly associated with PHA-induced wattle swelling in chicken, but only in the later phases of the response (≥ 72 h post-injection). This delay may reflect the initiation of T-cell proliferation (Martin *et al.* 2006).

Another explanation arises from the inclusion of non-additive genetic effects (dominance and maternal/early rearing effects) in the nest of origin effect that we detected. Recently, Pitala *et al.* (2007) used an animal model to distinguish additive from non-additive genetic effects on the response to PHA in collared flycatchers (*Ficedula albicollis*) and failed to show any evidence of a narrow sense heritable response to PHA. If PHA-induced swelling is mostly dominated by maternal effects, it is not surprising that our heritability estimate of nestling response to PHA, which includes early maternal influences, could not be explained by genetic variation.

We previously showed an association between the *Mhc* allele *a172* and response to PHA in this species (Bonneaud *et al.* 2005). However, because this association is unlikely to reflect a direct involvement of MHC molecules in the physiological processes elicited by PHA (Licastro *et al.* 1993; Bonneaud *et al.* 2005), the pattern we detected here could also result if other genes have stronger effects on this trait. In accordance with this hypothesis, responsiveness to PHA has been found to involve genes that are not tightly linked to the *Mhc* (Gasser *et al.* 1978; Morrow & Abplanalp 1981).

In any case, we cannot eliminate the possibility that, given our sample size, we failed to detect small effects. In fact, we only have 80 per cent power to detect an allelic effect that explains 20 per cent or more of the genetic variance (Purcell *et al.* 2003). Our study underscores the need for a precise examination of the role of genetic and environmental factors in the transmission of resistance and immunocompetence between generations, in order to gain a better understanding of how individuals optimize the fitness of their offspring.

Boerwinkle, E., Chakraborty, R. & Sing, C. F. 1986 The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. *Ann. Hum. Genet.* **50**, 181–194. (doi:10.1111/j.1469-1809.1986.tb01037.x)

Bonneaud, C., Sorci, G., Morin, V., Westerdahl, H., Zoorob, R. & Wittzell, H. 2004 Diversity of *Mhc* class I and IIB genes in house sparrows (*Passer domesticus*). *Immunogenetics* **55**, 855–865. (doi:10.1007/s00251-004-0648-3)

Bonneaud, C., Richard, M., Faivre, B., Westerdahl, H. & Sorci, G. 2005 An *Mhc* class I allele associated to the expression of T-dependent immune response in the house sparrow. *Immunogenetics* **57**, 782–789. (doi:10.1007/s00251-005-0046-5)

Bonneaud, C., Perez-Tris, J., Federici, P., Chastel, O. & Sorci, G. 2006 Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution* **60**, 383–389.

Chilson, O. P. & Kellychilson, A. E. 1989 Mitogenic lectins bind to the antigen receptor on human-lymphocytes. *Eur. J. Immunol.* **19**, 389–396. (doi:10.1002/eji.1830190225)

Cichón, M., Sendecka, J. & Gustafsson, L. 2006 Genetic and environmental variation in immune response of collared flycatcher nestlings. *J. Evol. Biol.* **19**, 1701–1706. (doi:10.1111/j.1420-9101.2006.01110.x)

De Neve, L., Soler, J. J., Perez-Contreras, T. & Soler, M. 2004 Genetic, environmental and maternal effects on magpie nestling-fitness traits under different nutritional conditions: a new experimental approach. *Evol. Ecol. Res.* **6**, 415–431.

Gasser, D. L., Winters, B. A. & Balabanmalenbaum, G. 1978 Evidence for a gene in rats affecting lymphocyte responsiveness to PHA. *Immunogenetics* **7**, 149–154. (doi:10.1007/BF01844000)

Gur, H., Geppert, T. D., Wacholtz, M. C. & Lipsky, P. E. 1999 The cytoplasmic and the transmembrane domains are not sufficient for class I MHC signal transduction. *Cell. Immunol.* **191**, 105–116. (doi:10.1006/cimm.1998.1417)

Kilpimaa, J., Van de Casteele, T., Jokinen, I., Mappes, J. & Alatalo, R. V. 2005 Genetic and environmental variation in antibody and T-cell mediated responses in the great tit. *Evolution* **59**, 2483–2489.

Kimball, P., Verbeke, S. & Shiffman, M. 2002 Proliferative suppression and class I insufficiency on peripheral blood cells from hepatitis C patients overcome by exogenous cytokines or high-dose mitogen. *Clin. Immunol.* **103**, 317–323. (doi:10.1006/clim.2002.5217)

Kuno, M., Goronzy, J., Weyand, C. M. & Gardner, P. 1986 Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper Tlymphocytes. *Nature* **323**, 269–273. (doi:10.1038/323269a0)

Lange, K., Cantor, R., Horvath, S., Perola, M., Sabatti, C., Sinsheimer, J. & Sobel, E. 2001 MENDEL version 4.0: a complete package for the exact genetic analysis of discrete traits in pedigree and population data sets. *Am. J. Hum. Genet.* **69**, 1886.

Lange, K., Sinsheimer, J. S. & Sobel, E. 2005 Association testing with MENDEL. *Genet. Epidemiol.* **29**, 36–50. (doi:10.1002/gepi.20073)

Licastro, F., Davis, L. J. & Morini, M. C. 1993 Lectins and superantigens membrane interactions of these compounds with T-lymphocytes affect immune responses. *Int. J. Biochem.* **25**, 845–852. (doi:10.1016/0020-711X(93)90239-B)

Lillehoj, H. S., Kim, C. H., Keeler, C. L. & Zhangt, S. 2007 Immunogenomic approaches to study host immunity to enteric pathogens. *Poult. Sci.* **86**, 1491–1500.

Martin, L. B., Han, P., Lewittes, J., Kuhlman, J. R., Klasing, K. C. & Wikelski, M. 2006 Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoecological technique. *Funct. Ecol.* **20**, 290–299. (doi:10.1111/j.1365-2435.2006.01094.x)

Morrow, P. R. & Abplanalp, H. 1981 Genetic control of lymphocyte-T mitogenesis in chickens. *Immunogenetics* **13**, 189–200. (doi:10.1007/BF00350785)

Mousseau, T. A. & Roff, D. A. 1987 Natural-selection and the heritability of fitness components. *Heredity* **59**, 181–197. (doi:10.1038/hdy.1987.113)

Pitala, N., Gustafsson, L., Sendecka, J. & Brommer, J. E. 2007 Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers. *Biol. Lett.* **3**, 418–421. (doi:10.1098/rsbl.2007.0135)

Purcell, S., Cherny, S. S. & Sham, P. C. 2003 Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**, 149–150. (doi:10.1093/bioinformatics/19.1.149)

Rubini, M., Selvatici, R., Orlando, P., Balugani, S., Balboni, A. & Gandini, E. 1992 Altered proliferative kinetics in PHA-activated human lymphocytes-T treated with the anti-HLA class-I monoclonal-antibody 01.65.

Cell Prolif. **25**, 405–414. (doi:10.1111/j.1365-2184.1992.tb01450.x)

Self, S. G. & Liang, K. Y. 1987 Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *J. Am. Stat. Assoc.* **82**, 605–610. (doi:10.2307/2289471)

Sorci, G., Moller, A. P. & Boulinier, T. 1997 Genetics of host-parasite interactions. *Trends Ecol. Evol* **12**, 196–200. (doi:10.1016/S0169-5347(97)01056-2)

Sundaresan, N. R. *et al.* 2005 Differential expression of inducible nitric oxide synthase and cytokine mRNA in chicken lines divergent for cutaneous hypersensitivity response. *Vet. Immunol. Immunopathol.* **108**, 373–385. (doi:10.1016/j.vetimm.2005.06.011)

Taylor, R. L., Cotter, P. F., Wing, T. L. & Briles, W. E. 1987 Major histocompatibility (b) complex and sex effects on the phytohemagglutinin wattle response. *Anim. Genet.* **18**, 343–350.