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*Biol. Lett.*, published online 11 August 2009
doi: 10.1098/rsbl.2009.0435

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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 nesting 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 nesting 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.

**Keywords:** major histocompatibility complex; phytohaemagglutinin-induced skin swelling; cross-fostering; heritability; house sparrow

1. INTRODUCTION

Genetic contributions to the phenotypic variability of parasite resistance and immune response have mainly been demonstrated in domestic or laboratory species (Lilliehoj 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 immunological 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 & Kellycholson 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 response 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 nesting 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’Étude 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 sampled 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 MÉNDEL (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.)

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

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.)

<table>
<thead>
<tr>
<th>allele frequency</th>
<th>estimate</th>
<th>s.e.</th>
<th>CV</th>
<th>$p$-value</th>
<th>residual $h^2$ (s.e.)</th>
<th>% heritability explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of $Mhc$ alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Mhc$ allele a158</td>
<td>0.50</td>
<td></td>
<td>10.44</td>
<td>1.40</td>
<td>0.22</td>
<td>0.46 (0.15)</td>
</tr>
<tr>
<td>$Mhc$ allele a172</td>
<td>0.28</td>
<td></td>
<td>10.54</td>
<td>0.71</td>
<td>0.08</td>
<td>0.43 (0.16)</td>
</tr>
<tr>
<td>$Mhc$ allele a161</td>
<td>0.23</td>
<td></td>
<td>12.46</td>
<td>1.67</td>
<td>0.27</td>
<td>0.46 (0.15)</td>
</tr>
<tr>
<td>$Mhc$ allele a151</td>
<td>0.22</td>
<td></td>
<td>12.49</td>
<td>14.27</td>
<td>0.47</td>
<td>0.44 (0.17)</td>
</tr>
<tr>
<td>$Mhc$ allele a165</td>
<td>0.21</td>
<td></td>
<td>12.32</td>
<td>1.09</td>
<td>0.18</td>
<td>0.48 (0.15)</td>
</tr>
<tr>
<td>$Mhc$ allele a163</td>
<td>0.20</td>
<td></td>
<td>12.70</td>
<td>0.78</td>
<td>0.10</td>
<td>0.41 (0.16)</td>
</tr>
<tr>
<td>$Mhc$ allele a123</td>
<td>0.01</td>
<td></td>
<td>10.67</td>
<td>0.63</td>
<td>0.06</td>
<td>0.39 (0.16)</td>
</tr>
</tbody>
</table>

Environmental ($V_H$), shared nest of rearing environmental/household ($V_M$), 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 $V_A$, $V_H$ and $V_M$, so that only $V_H$ is included in the model. Heritability was calculated as $h^2 = V_A / V_H$, where $V_H$ 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 without and without the additive effect (see the electronic supplementary material, methods; Self & Liang 1987). The statistical significance of the rearing environment ($V_M$) 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. 2003) and by incorporating each factor separately into the model. Statistical significance was determined using a standard normal $Z$-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 (Cichon 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.

All procedures conducted in this study adhered to guidelines stipulated by laws of animal research in the country in which the research was undertaken and to those outlined by ASAB in their ethics statement on the use of animals for scientific purposes.

We thank Miguel Alcaide and Andy Russell for useful comments and Celine Chastel for help in the field. Financial support came from the Ministère de la Recherche (bourse doctorale to C.B. and ACI Jeunes Chercheurs to G.S.; France), from l’Agence Nationale de la Recherche (ANR-05-BLAN-0265-01; France) and from the United States Public Health Service grants MH59490 and GM53275.


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