Inbreeding depresses immune response in song sparrows (Melospiza melodia): direct and inter-generational effects

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A thorough knowledge of relationships between host genotype and immunity to parasitic infection is required to understand parasite-mediated mechanisms of genetic and population change. It has been suggested that immunity may decline with inbreeding. However, the relationship between inbreeding level and a host’s response to a novel immune challenge has not been investigated in a natural population. We used the pedigreed population of song sparrows (Melospiza melodia) inhabiting Mandarte Island, Canada, to test the hypothesis that a sparrow’s cell-mediated immune response (CMI) to an experimental challenge would decline with individual or parental inbreeding. CMI in 6-day-old chicks declined significantly with their mother’s coefficient of inbreeding, demonstrating an inter-generational effect of maternal inbreeding on offspring immunity. In fledged juveniles and adult sparrows, CMI declined markedly with an individual’s own coefficient of inbreeding, but not its mother’s. This relationship was consistent across seasons, and was not attributable solely to heterosis in offspring of immigrant breeders. CMI also declined with age and increased with body condition in adult sparrows, but inbreeding explained 37% of the total variation. We emphasize the implications of this dramatic inbreeding depression in cell-mediated immunity for theories of parasite-mediated evolution and the susceptibility of small, inbred populations.

Keywords: cell-mediated immunity; conservation genetics; maternal effect; parasite-mediated evolution; senescence

1. INTRODUCTION

Interactions between host genotype and immunity to parasitic infection have been suggested to underlie major evolutionary processes. For example, fluctuating selection resulting from rapid parasite evolution may facilitate the maintenance of host genetic diversity, while honest sexual signalling may be maintained by genotype-specific costs of immunity (Hamilton & Zuk 1982; Potts & Wakeland 1990). Comprehensive development of such theories requires a clear understanding of the genetic basis of immunity, and the extent to which genetic effects are modulated by current and previous environments.

To date, most theoretical and empirical studies have focused on additive genetic influences on immunity. Theories of parasite-mediated evolution generally assume that immunity is heritable (Sorci et al. 1997a). Breeding experiments on laboratory and domesticated vertebrates support this assumption, and loci involved in mediating parasite resistance have been identified (Wakelin 1996; Carrington et al. 1999). Immunity is also heritable in wild vertebrate populations (Sorci et al. 1997a; Smith et al. 1999; Svensson et al. 2001). However, offspring immunity is often influenced more by early environmental or maternal effects than any additive genetic component (Sorci et al. 1997a; Brinkhof et al. 1999; Christe et al. 2000; Tella et al. 2000). This may reflect the immunological consequences of the quality of early nutrition, or direct transfer of maternal antibodies or enzymes (Lochmiller et al. 1993; Gasparini et al. 2001; Saino et al. 2002b). However, it is not clear to what extent natal effects on immunity persist throughout an individual’s lifetime, or whether these effects may themselves reflect aspects of parental genotype.

It has also been suggested that host immunity may decline with inbreeding (O’Brien & Evermann 1988). This hypothesis is plausible since inbreeding increases homozygosity (Falconer & Mackay 1996) and immunity can decline with homozygosity (such as at major histocompatibility complex (MHC) loci; Potts & Wakeland 1990; Carrington et al. 1999). Inbreeding, however, affects phenotypic traits through dominance rather than additive genetic effects, and in sexually reproducing organisms dominance effects are not directly heritable (narrow-sense heritability; Falconer & Mackay 1996). A decline in immunity with inbreeding would therefore imply a genetic effect on immunity that differs fundamentally from additive genetic mechanisms. The overall effects of parasites on patterns of genetic change could consequently differ from those envisaged assuming solely additive genetic effects on immunity. Further, as parasitic infections can alter population trajectories and cause extinctions (McCallum & Dobson 1995), a decline in immunity with inbreeding would have implications for the dynamics and persistence of small and isolated populations in which inbreeding is likely.

The parasites that wild populations experience, and that may thus drive genetic and population change, are expected to be diverse and to change over time (Hedrick...
et al. 2001; Lafferty & Gerber 2002). New, emergent diseases may exert particularly strong selection on naïve populations (Friend et al. 2001). To fully evaluate the evolutionary and ecological impacts of parasites on natural populations where inbreeding occurs, the relationship between inbreeding and a host’s response to a novel immune challenge should therefore be described. This relationship cannot, however, be inferred from existing data. Previous studies relating vertebrate immunity to inbreeding have used specific pairs of coexisting host and parasite species, and only one monitored a wild population (Table 1). While some studies showed that parasite resistance declined with inbreeding or homozygosity, this was not consistent across all hosts or all parasites (e.g. Cassinello et al. 2001; Arkush et al. 2002). Here, we investigate the relationship between inbreeding and the response to a novel immune challenge in a natural bird population.

Host immunity cannot be assessed by simply measuring parasite loads because observed infections reflect variation in both resistance and exposure. Experimental immune challenges, which eliminate confounding variation in exposure, provide a clearer measure of immunity (Norris & Evans 2000). Such techniques have been widely used to investigate the evolutionary ecology of immunity in the contexts of life-history allocation and sexual signaling (e.g. Deerenberg et al. 1997; Zuk & Johnsen 1998; Svensson et al. 2001), but have not previously been applied to inbreeding. Using the pedigreed population of song sparrows (Melospiza melodia) inhabiting Mandarte Island, British Columbia, Canada, we assessed a sparrow’s cell-mediated immune response to a novel, experimental challenge. We hypothesized that this response might decline with levels of individual or parental inbreeding, and investigated whether the effects of parental inbreeding on immunity remained after offspring reached independence from parental care.

2. MATERIAL AND METHODS

(a) Study population

The resident Mandarte Island song sparrow population, which numbered 25 breeding pairs in 2002, has been studied intensively since 1975. All individuals born on the island, plus the occasional immigrants to the breeding population, have been individually colour-ringed, and their subsequent life histories closely monitored. The resulting social pedigrees allow individual coefficients of inbreeding to be estimated (Wright’s f; see Keller 1998; Marr et al. 2002). The f-value reflects the probability that two homologous alleles will be identical by descent (Falconer & Mackay 1996), and currently averages 0.06 across the Mandarte population (approximately equivalent to a first-cousin pairing; see also Keller 1998). While immigrants are themselves of unknown inbreeding status, they are likely to be unrelated to the Mandarte population (Keller et al. 2001). Their offspring are therefore assumed to be outbred (f = 0; Keller 1998; Marr et al. 2002). Adult survival and lifetime reproductive performance decline with inbreeding in this population (Keller 1998).

(b) Cell-mediated immunity

Cell-mediated immunity (CMI) is one component of the avian acquired immune system, acting primarily against intra-
cellular bacterial, viral and fungal infections (Wakelin 1996). We measured a sparrow’s CMI as the wing-web (patagium) swelling response to subcutaneous injection of phytohaemagglutinin (PHA; Goto 1997; Polak et al. 1996). PHA is a non-specific mitogen, and subcutaneous injection induces a perivascular accumulation of leucocytes. We measured CMI in adult (post-natal year) sparrows between 16 and 24 February 2002 (hereafter February 2002) and in adult and juvenile (natal year) sparrows between 22 September and 3 October 2002 (September 2002). Juveniles were between four and five months old at this time, and were no longer associated with their parents or natal territories. As measuring CMI requires two handling occasions separated by a standardized interval and we could not reliably recapture free-flying birds, we chose these seasons because adults could be retained in captivity over the experimental period without affecting breeding activities. Sparrows were mist-netted after mid-morning, and wing length, tarsus length and mass were recorded. The left and right patagial thicknesses were measured three times using a modified dial caliper accurate to 0.01 mm (Mitutoyo, Japan). Sparrows were injected with 30 µl of 2 mg ml\(^{-1}\) PHA (L9132, Sigma, St Louis, MO, USA) in phosphate buffered saline (PBS, 9.7 g l\(^{-1}\) D5773, Sigma, St Louis, MO, USA) in the right patagium and 30 µl of PBS in the left patagium as a control, provided with ad libitum food and water, and roosted overnight in individual enclosures. The right and left patagial thicknesses were remeasured three times the following morning, ca. 18 h after injection. Sparrows were then reweighed and released. CMI was estimated as the difference in the mean thickness change of the right and left patagia over the experimental period: a higher score indicates a stronger immune response.

Between 5 May and 30 June 2002 (spring 2002), we measured CMI in all first and second brood chicks that survived to 60 days old. Nests were located during incubation then visited between 14.30 and 17.30 5 days after chicks hatched. Patagial thicknesses were measured three times, and chicks were injected with 20 µl of 2 mg ml\(^{-1}\) PHA in PBS and 20 µl of PBS in the right and left patagia, respectively, and returned to the nest. Patagia were remeasured 24 h later and CMI was calculated as for adults. Wing length, tarsus length and mass were recorded during both nest visits. For all ages, patagial thickness was highly repeatable across each set of three repeat measurements on each individual (\(r > 0.94, p < 0.0001\)). Error propagation calculations indicated that the degree of uncertainty that measurement error introduced into CMI estimates was therefore relatively small (less than 5% based on the sum of average deviations; Harper & Weaire 1985). All data were collected with no knowledge of individual or parental inbreeding coefficients.

(c) Analysis

We used general linear mixed models to identify variables that explained a significant proportion of the variation in CMI. As siblings may not be independent with respect to their immune response (Saino et al. 1997; Sorci et al. 1997b), we modelled brood and parent identities as random factors (PROC MIXED, SAS Institute). While our primary aim was to examine the relationships between CMI and individual and parental inbreeding, we included age, sex, body condition and date; previous studies have shown immunological effects of these variables (e.g. Saino et al. 1997; Brinkhof et al. 1999; Alonzo-Alvarez & Tella 2001). Adult sexes were determined by observing breeding behaviour. Using a discriminant function based on wing length, we confidently assigned sexes to 23 out of 35 juveniles caught during September 2002 (\(p > 0.95\)). Body condition was estimated as the residual of mass on the cube of the first principal component of wing and tarsus length, calculated separately for juveniles and each adult sex. Dependent variables were log-transformed to correct for heteroscedasticity and deviations from normality where necessary. Independent variables were not significantly correlated except where mentioned below.

On Mandarte, the only fledged sparrows currently considered completely outbred \((f = 0)\) are six offspring of three immigrant breeders. Depending on the degree of genetic divergence between populations, these offspring could show elevated performance due to heterosis (Falconer & Mackay 1996), as suggested on Mandarte (Marr et al. 2002). To investigate whether relationships between \(f\) and CMI were due to inbreeding among Mandarte-born sparrows rather than solely heterosis in immigrants’ offspring, we repeated analyses after excluding these individuals.

### 3. RESULTS

(a) Adults

We measured CMI in 25 adult song sparrows in February 2002, and 18 adults (including six that were also tested in February) in September 2002. The total of 37 adults included nine sets of two or more siblings. Ages ranged from \(ca. 1\) to 8 years (median two), and \(f\) ranged from 0 to 0.18 (mean 0.06). In February, CMI declined with individual \(f\) (figure 1a). In September, CMI did not differ between adults that had and had not been tested in February \((p = 0.97)\), and again declined with individual \(f\) (figure 1a). No other covariates explained a significant proportion of the variation during either individual season. Across both seasons combined, CMI varied significantly with \(f\), age and body condition: sparrows responded weakly to the immune challenge when inbred, old and in poor condition (table 2). Inbreeding accounted for 37% of the total variation, and no sibling effect was detected. To maintain data independence in the combined analysis, only the February (first exposure) data were included from the six adults that were tested twice.

(b) Chicks

During spring 2002, we measured CMI in 94 chicks comprising 36 first and second broods of 24 females and 25 pairings (one female switched to a previously unmated male between broods). Across all females, maternal \(f\) was correlated with offspring \(f\) (\(r = 0.54\)). However, this relationship was greatly influenced by one particularly inbred female who produced inbred offspring. Excluding this brood (the first of the female that subsequently switched males) substantially weakened the correlation (\(r = 0.28\)). To check whether collinearity affected our conclusions, we repeated analyses after excluding this brood. After accounting for random brood and parental effects, chick CMI declined with maternal \(f\) (figure 1b). No other covariates explained a significant proportion of the residual variation (table 2). Excluding the inbred brood did not alter the final model, although there was an increased tendency for chicks that were themselves inbred to respond more weakly to the immune challenge (\(p = 0.09\)).
During September 2002, we remeasured CMI in 35 fledged juvenile sparrows (37% of the 94 chicks tested during Spring 2002) and one offspring of 22 of the 24 females that bred successfully during 2002 and two or more offspring of nine females.

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(c) Juveniles

During September 2002, we remeasured CMI in 35 fledged juvenile sparrows (37% of the 94 chicks tested during Spring 2002). These 35 included at least one offspring of 22 of the 24 females that bred successfully during 2002 and two or more offspring of nine females. 

Figure 1. Relationships between (a) an adult song sparrow’s coefficient of inbreeding (f) and its CMI response; (b) a female song sparrow’s coefficient of inbreeding (f) and CMI response in her 6-day-old chicks; and (c) a juvenile song sparrow’s coefficient of inbreeding (f) and CMI response. Adult CMI response declined significantly with f in both February (filled circles, F1,25 = 4.2, p = 0.05, R2 = 0.15) and September 2002 (open circles, F1,19 = 30.8, p < 0.001, R2 = 0.66). For simplicity, (b) shows sibling means. CMI response declined significantly with maternal f in chicks, but with an individual’s own f in juveniles (table 2). A moderate degree of individual or maternal inbreeding (f = 0.1) resulted in a reduction in individual or chick CMI response of ca. 40%.

Table 2. Models predicting variation in CMI in adult (post-natal year) song sparrows, 5–6-day-old chicks and juvenile (1–5-month-old) song sparrows.

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<tr>
<th>covariates</th>
<th>final model</th>
<th>random factors</th>
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<td>n</td>
<td>F2,5 = 36.2</td>
<td>F1,9 = 1.9</td>
<td>F1,5 = 0.001</td>
<td>F1,22</td>
<td>F1,8</td>
<td>F1,5 = 0.007</td>
<td>p = 0.18</td>
<td>p = 0.004</td>
<td>p = 0.007</td>
<td>p = 0.001</td>
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<tr>
<td>chicks</td>
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<td>F1,9 = 0.95</td>
<td>F1,5 = 0.84</td>
<td>F1,22</td>
<td>F1,8</td>
<td>F1,5 = 0.007</td>
<td>p = 0.18</td>
<td>p = 0.004</td>
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<td>F1,9 = 0.82</td>
<td>F1,5 = 0.007</td>
<td>F1,22</td>
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<td>F1,5 = 0.007</td>
<td>p = 0.18</td>
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residual variation (table 2). However, across these same 35 individuals, their CMI as chicks had declined significantly with their mother’s coefficient of inbreeding ($p = 0.003$).

(d) Heterosis

Declines in CMI with individual $f$ in adults and juveniles, and with maternal $f$ in chicks, remained significant when offspring of immigrant breeders were excluded (all $p < 0.004$). Parameter estimates changed by only 4% on average, suggesting that any heterosis in these offspring did not exacerbate observed relationships between $f$ and CMI.

4. DISCUSSION

After accounting for random variation in the familial environment, CMI in song sparrow chicks declined with maternal (but not paternal) inbreeding; offspring of inbred females showed a weaker immune response. While chick immunity often depends more on the natal environment than additive genetic variation (Sorci et al. 1997a), our results suggest that natal effects may themselves vary with non-additive properties of the maternal genome. As inter-generational maternal effects can influence population dynamics and rates of evolutionary change (Wolf et al. 1998; Beckerman et al. 2002), these effects should be incorporated into future assessments of the conservation and evolutionary implications of inbreeding.

In juvenile and adult sparrows, CMI declined with an individual’s own coefficient of inbreeding and not its mother’s. This relationship persisted when offspring of immigrants were excluded, and can therefore be attributed to inbreeding among Mandarte-born sparrows rather than solely heterosis in outbred progeny of between-population crosses. The response to a novel immune challenge therefore declined clearly and consistently with inbreeding in free-living song sparrows. CMI also declined with increasing age in adults, suggesting immunological senescence that has rarely been reported in wild vertebrates (see Saino et al. 2002a; Lozano & Lank 2003).

In poultry, the cell-mediated response to PHA injection shows additive genetic variance and has been linked to MHC loci (Taylor et al. 1987; Warner et al. 1987; Cheng & Lamont 1988). Inbreeding may therefore affect CMI by increasing expression of deleterious alleles or reducing overdominance at crucial response loci. However, mounting an immune response may be energetically demanding (Deerenberg et al. 1997; Ots et al. 2001, although see Råberg et al. 1998) and dependent upon an individual’s nutritional and hormonal state (Lochmiller et al. 1993; Alonzo-Alvarez & Tella 2001; Casto et al. 2001). Inbreeding may therefore affect CMI through more general effects on metabolic or endocrinological pathways. Although adult sparrows in poor condition showed reduced CMI, body condition did not decline directly with inbreeding ($p = 0.25$). However, the transient effect of maternal inbreeding on offspring CMI suggests a nutritional mechanism; inbred mothers may have been less able to provision eggs or chicks. Chicks of inbred mothers tended to gain less mass over the immunoassay period ($p = 0.10$), although CMI did not vary directly with chick condition ($p = 0.50$). The absence of an effect of paternal inbreeding on chick CMI may reflect the lesser contribution of males to early parental care in song sparrows.

We studied the relationship between inbreeding and one component of avian immunity. Since different components do not necessarily show correlated responses, it would be valuable to investigate inbreeding effects on others (Norris & Evans 2000). Nevertheless, the marked inbreeding depression in CMI has several major implications. Given exposure to relevant parasites, more inbred individuals may be more susceptible to infection (Warner et al. 1987; Roitt et al. 1996; Nordling et al. 1998). As infections can reduce host survival and fecundity, our results suggest one proximate ecological mechanism causing inbreeding depression in these traits (Keller 1998), and support the notion that parasite-mediated selection against inbred (and thus more homozygous) individuals might maintain genetic diversity within a population (Paterson et al. 1998; Coltman et al. 1999). Although links between immune response and fitness require further quantification (Norris & Evans 2000), the decline in CMI that we observed with moderate inbreeding ($f = 0.1$; figure 1) was of similar magnitude to that which predicted reduced survival in house sparrows (Passer domesticus; Gonzalez et al. 1999b).

In addition, the small and isolated populations in which inbreeding is likely may be more susceptible to infection. While bottlenecks may increase population susceptibility by increasing loss of resistant alleles by drift (O’Brien & Evermann 1988), susceptibility may be further exacerbated if inbreeding occurs after a population decline. It has been suggested that populations of conservation concern could be protected from parasites by reducing immigration (Hess 1996). However, although we found no additional effect of heterosis over that of outbreeding within Mandarte, the offspring of immigrants to Mandarte (attributed $f = 0$) and their chicks showed relatively strong CMI (figure 1). Our results therefore suggest that immigration may increase an inbred population’s ability to respond to a novel immune challenge.

Finally, secondary sexual traits have been proposed to signal a male’s genetic resistance to parasites (Hamilton & Zuk 1982; Folstad & Karter 1992). Consistent with this hypothesis, ornament size or complexity has been shown to correlate positively with immune function in multiple systems, including with CMI in birds (Zuk & Johnsen 1998; Gonzalez et al. 1999a; Duffy & Ball 2002; Møller & Petrie 2002; Saino et al. 2002c). Given that in adult song sparrows inbreeding in turn explained 37% of variation in CMI, the possibility that ornaments may to some extent signal inbreeding deserves further theoretical and empirical consideration.

The Tsawout and Tseycum First Nations bands allowed the authors to work on Mandarte. Danielle Dagenais, Steven Gates, Jamie Smith, Jason Weir and Scott Wilson provided invaluable assistance with fieldwork. Harpreet Gill, Amy Marr and Carol Ritland assisted with protocols and analyses. Elizabeth Gillis, Hanna Kokko, Pat Monaghan, Jamie Smith, Mike Stear and three anonymous referees gave constructive advice on the manuscript. Fieldwork was approved by the UBC Animal Care Committee and funded by the British Ecological Society, University of Glasgow Ferguson Bequest Fund, Glasgow Natural History Society and NSERC. J.M.R. is supported by the Natural Environment Research Council and the University of Glasgow.
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Inbreeding and immunity

J. M. Reid and others


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