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

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et al. 2001; Lafferty & Gerber 2002). New, emergent diseases may exert particularly strong selection on naive 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 signalling (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 tested the hypothesis 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 pedigree allows 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-

Table 1. Studies that examined relationships between host inbreeding and resistance to parasitic infection in vertebrates.

study	host	parasites	system	measure of inbreeding	measure of response	relationship
Arkush <i>et al.</i> (2002)	chinook salmon (<i>Oncorhynchus tshawytscha</i>)	<i>Listonella anguillarum</i> whirling disease (<i>Myxobolus cerebralis</i>)	laboratory	inbred lines, MHC heterozygosity	prevalence of infection, intensity of infection, host survival	<i>Myxobolus</i> infection increased with inbreeding, HPNV increased with homozygosity (7/10 families), otherwise no relationship
Cassinello <i>et al.</i> (2001)	Cuvier's gazelle (<i>Gazella cuvieri</i>) dama gazelle (<i>Gazella dama</i>) dorcass gazelle (<i>Gazella dorca</i>)	haematopoietic necrosis virus <i>Nematodirus</i> spp. <i>Trichouris</i> spp.	captivity	pedigree coefficient of inbreeding	intensity of infection	infection increased with inbreeding in <i>G. cuvieri</i> but not <i>G. dama</i> or <i>G. dorca</i>
Coltman <i>et al.</i> (1999)	Soay sheep (<i>Ovis aries</i>)	gastrointestinal nematodes (<i>Teladorsagia circumcincta</i>)	wild	microsatellite heterozygosity	intensity of infection	infection increased with homozygosity (adults only)
Ferguson & Drahushchak (1990)	rainbow trout (<i>Oncorhynchus mykiss</i>)	bacterial gill disease (<i>Flavobacterium</i> spp.)	laboratory	enzyme heterozygosity	host survival	survival decreased with homozygosity
Hedrick <i>et al.</i> (2001)	gila topminnow (<i>Poeciliopsis occidentalis</i>)	<i>Gyrodactylus turnbulli</i>	laboratory	inbred lines, MHC heterozygosity	prevalence of infection, intensity of infection, host survival	infection increased with inbreeding (although <i>p</i> > 0.05), but not homozygosity

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 *et al.* 1978; Roitt *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 patagium thicknesses were measured three times using a modified dial caliper accurate to 0.01 mm (Mitutoyo, Japan). Sparrows were injected with 30 μ l 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 μ l 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 6 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 μ l of 2 mg ml⁻¹ PHA in PBS and 20 μ l 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, patagia 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 ($p = 0.09$).

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, $F_{1,25} = 4.2$, $p = 0.05$, $R^2 = 0.15$) and September 2002 (open circles, $F_{1,18} = 30.8$, $p < 0.001$, $R^2 = 0.66$). 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%.

(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. f ranged from 0 to 0.30 (mean 0.06). Across the 23 juveniles of known sex, CMI did not differ between males and females ($p = 0.26$). Data from all 35 juveniles were therefore pooled. CMI declined with a juvenile's own inbreeding coefficient (figure 1c). No other covariates, including maternal f ($p = 0.49$) or the random sibling effect ($p = 0.19$) explained a significant proportion of the

Table 2. Models predicting variation in CMI in adult (post-natal year) song sparrows, 5–6-day-old chicks and juvenile (4–5-month-old) song sparrows. (Covariates retained in final models are indicated in bold.)

		covariates					fixed factors				random factors		
		individual's inbreeding coefficient	mother's inbreeding coefficient	father's inbreeding coefficient	initial body condition	growth during assay	age	assay date	sex	field period	brood	parents	final model
adults	37	$F = -36.2$ $p < 0.001$	$F = -1.9$ $p = 0.18$	$F = -0.22$ $p = 0.64$	$F = 8.2$ $p = 0.007$	—	$F = -4.4$ $p = 0.04$	$F = 0.1$ $p = 0.81$	$F = 2.9$ $p = 0.10$	$F = 0.1$ $p = 0.80$	—	$Z = 0.1$ $p = 0.95$	$F_{3,36} = 13.5, p < 0.001,$ $R^2 = 0.56$
chicks	94	$F = -1.4$ $p = 0.25$	$F = -12.8$ $p = 0.002$	$F = -1.7$ $p = 0.21$	$F = 0.5$ $p = 0.50$	$F = 0.5$ $p = 0.50$	—	$F = 1.2$ $p = 0.29$	—	—	$Z = 1.9$ $p = 0.03$	$Z = 1.4$ $p = 0.08$	$F_{1,165} = 12.8, p = 0.002,$ $R^2 = 0.47$
juveniles	35	$F = -23.1$ $p < 0.001$	$F = -0.5$ $p = 0.49$	$F = -1.2$ $p = 0.29$	$F = 2.0$ $p = 0.17$	—	—	$F = 0.5$ $p = 0.50$	—	—	—	$Z = 0.9$ $p = 0.19$	$F_{1,35} = 23.1, p < 0.001,$ $R^2 = 0.41$

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.

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