Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (*Melospiza melodia*)

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Hamilton and Zuk's influential hypothesis of parasite-mediated sexual selection proposes that exaggerated secondary sexual ornaments indicate a male's additive genetic immunity to parasites. However, genetic correlates of ornamentation and immunity have rarely been explicitly identified. Evidence supporting Hamilton and Zuk's hypothesis has instead been gathered by looking for positive phenotypic correlations between ornamentation and immunity; such correlations are assumed to reflect causal, additive relationships between these traits. We show that in song sparrows, *Melospiza melodia*, a male's song repertoire size, a secondary sexual trait, increased with his cell-mediated immune response (CMI) to an experimental challenge. However, this phenotypic correlation could be explained because both repertoire size and CMI declined with a male's inbreeding level. Repertoire size therefore primarily indicated a male's relative heterozygosity, a non-additive genetic predictor of immunity. Caution may therefore be required when interpreting phenotypic correlations as support for Hamilton and Zuk's additive model of sexual selection. However, our results suggest that female song sparrows choosing males with large repertoires would on average acquire more outbred and therefore more heterozygous mates. Such genetic dominance effects on ornamentation are likely to influence evolutionary trajectories of female choice, and should be explicitly incorporated into genetic models of sexual selection.

Keywords: cell-mediated immunity; good genes; inbreeding depression; parasite-mediated selection; secondary sexual ornament

1. INTRODUCTION

Genetic theories of intersexual selection propose that exaggerated secondary sexual ornaments indicate some aspect of a male's genetic quality. Females might then benefit from choosing more ornamented males by acquiring 'good genes' for their offspring (Kirkpatrick & Ryan 1991; Andersson 1994; Kokko *et al.* 2002, 2003). The precise nature of these good genes must be described in order to understand how genetic systems of sexual selection might evolve and be maintained. However, although these issues have received considerable theoretical attention, genetic correlates of ornamentation have rarely been explicitly identified (Andersson 1994; Brown 1997; Siva-Jothy & Skarstein 1998).

Many models propose that 'good genes' comprise specific alleles that enhance performance compared to homologous alleles, and therefore confer additive fitness benefits (Andersson 1994; Kokko *et al.* 2003). Consistent with these models, ornaments have been shown to indicate heritable components of fitness (e.g. Norris 1993; Welch *et al.* 1998). Yet, despite this empirical evidence, the notion that females choose males for additive genetic

benefits is difficult to defend as a theoretical possibility. This is because directional sexual selection is predicted to deplete additive genetic variance for fitness, eliminating the benefit of female choice (Kirkpatrick & Ryan 1991; Andersson 1994; Kokko *et al.* 2003). Additional mechanisms maintaining genetic variance are therefore required.

One such mechanism, first proposed by Hamilton & Zuk (1982), is that 'good genes' confer additive genetic immunity to parasites. Genetic variance could then be maintained by cycles of host–parasite coevolution (Hamilton & Zuk 1982). This influential hypothesis has become central to good genes theories of sexual selection, and has prompted considerable research into the nature and basis of relationships between ornamentation and immunity (Folstad & Karter 1992; Andersson 1994; Westneat & Birkhead 1998; Møller *et al.* 1999). However, one main prediction, that ornaments indicate a male's additive genetic immunity to parasites, has proved difficult to test. Three studies have investigated whether ornamentation indicates heritable variation in phenotypic immunity (Møller 1990; Kurtz & Sauer 1999; Svensson *et al.* 2001), while von Schantz *et al.* (1996) showed that spur length indicates MHC genotype in pheasants (*Phasianus colchicus*). Such direct tests are scarce because the genetic

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basis of immunity can rarely be identified. Most studies have instead measured correlations between ornamentation and some phenotypic index of infection or immunity (e.g. Møller *et al.* 1999; Ryder & Siva-Jothy 2000; Duffy & Ball 2002). This phenotypic approach provides a weak test of Hamilton and Zuk's hypothesis because the possibility of complex life-history trade-offs clouds the interpretation of apparently null results (Kokko *et al.* 2002). However, significantly positive phenotypic correlations are frequently interpreted as clear supporting evidence (reviewed by Møller *et al.* 1999). This interpretation assumes that positive correlations reflect causal, additive relationships between ornamentation and immunity. Few studies, however, have verified this assumption, or considered what other genetic mechanisms might be responsible.

More recently, 'good genes' have been suggested to comprise heterozygosity (Brown 1997). This definition differs fundamentally from the view that such genes are additive, beneficial alleles because heterozygosity is a non-additive genetic quantity (Falconer & Mackay 1996). The definition of 'good genes as heterozygosity' (Brown 1997) does, however, make intuitive sense because fitness often declines with homozygosity and inbreeding (Keller & Waller 2002). Females might therefore be expected to maximize heterozygosity in offspring, for example by allocating paternity to genetically diverse or dissimilar males (Landry *et al.* 2001; Tregenza & Wedell 2002; Foerster *et al.* 2003). Females may identify these diverse or dissimilar males using pheromones, or vocal or visual recognition (Brown & Eklund 1994; Landry *et al.* 2001). However, to integrate the notion of good genes as heterozygosity with the paradigm that females prefer more ornamented males, it is also possible that secondary sexual ornaments might themselves indicate some aspect of a male's own genetic diversity, such as his average heterozygosity (Brown 1997). Evolutionary trajectories of intersexual selection could in this case differ substantially from those predicted assuming female choice for additive good genes (Charlesworth 1988). Furthermore, heterozygosity is known to influence immunity (Potts & Wakeland 1990; Paterson *et al.* 1998). A link between male heterozygosity and ornamentation would therefore raise the possibility that phenotypic correlations between ornamentation and immunity, which are often interpreted as support for Hamilton and Zuk's additive model of parasite-mediated sexual selection, could in fact arise because both traits decline with homozygosity.

We used the pedigreed population of song sparrows *Melospiza melodia* inhabiting Mandarte Island, Canada, to investigate relationships between a male's song repertoire size, coefficient of inbreeding (f) and cell-mediated immune response (CMI). Song repertoire size is a classic example of an ornamental secondary sexual trait that is thought to influence female mate choice (Searcy 1992; Andersson 1994). Song sparrows provide one well-studied example. Males sing repertoires of between 4 and 13 distinct song types (Cassidy 1993; Nordby *et al.* 2002). Experimental and correlative evidence suggests that females prefer males with larger repertoires (Searcy & Marler 1981; Reid *et al.* 2004). First, we show that song repertoire size declined with a male's inbreeding coefficient and therefore indicated his relative heterozygosity. We used inbreeding coefficient as our measure of relative

genome-wide heterozygosity (Falconer & Mackay 1996) because f provides a more accurate estimate of this quantity than heterozygosity measured at a small number of marker loci (Slate *et al.* 2004). Second, we show that song repertoire size increased with a male's CMI and therefore indicated his phenotypic immunity. Finally, we show that the significant phenotypic correlation between ornamentation and immunity could be explained because both traits declined with f . We discuss the implications of these apparent genetic dominance effects on ornamentation and immunity for genetic models of intersexual selection, and interpretations of phenotypic correlations in the context of Hamilton & Zuk's (1982) hypothesis of parasite-mediated sexual selection.

2. MATERIALS AND METHODS

(a) *Study population*

Mandarte Island, six hectares in size, lies 25 km north-east of Victoria, British Columbia, Canada. Its resident and relatively isolated song sparrow population has been studied intensively since 1975, and has varied between 4 and 72 breeding pairs during this time. During the study, all song sparrows fledged on Mandarte, and the occasional immigrants to the population, have been individually colour-ringed. Detailed behavioural observations have allowed a social pedigree to be constructed, covering all sparrows hatched since 1981 (Keller 1998). These data allow individual coefficients of inbreeding (Wright's f , the probability that two homologous alleles will be identical by descent) to be calculated (Falconer & Mackay 1996; Keller 1998). While immigrants to Mandarte are themselves of unknown inbreeding status, comparison of microsatellite genotypes indicates that they are unrelated to the resident Mandarte population (Keller *et al.* 2001). Immigrants' offspring are therefore considered outbred ($f = 0$; Keller 1998; Marr *et al.* 2002). To reduce the chance that f was underestimated due to insufficient pedigree depth (Keller 1998), current analyses were restricted to individuals born after 1984. Although extra-pair paternities occur on Mandarte, there is no evidence that they occur systematically with respect to relatedness. Over 4 years, the frequency of extra-pair paternity did not vary with a female's relatedness to her social mate, and extra-pair sires were no more or less related to focal females than expected by chance (O'Connor *et al.* submitted). Extra-pair paternities are therefore likely to introduce error but not bias into estimates of inbreeding coefficients (f). Such errors are likely to cause us to underestimate, but not overestimate, inbreeding depression (Keller *et al.* 2002; Marr *et al.* in press; see also Cassell *et al.* 2003). The effect sizes that we report are therefore minimum estimates.

(b) *Song repertoire size*

Male song sparrows learn their song repertoire during their first few months of life, then retain the same songs for life (Cassidy 1993; Nordby *et al.* 2002). During 1988–1993 and 2003, we measured repertoire sizes of 53 male song sparrows, hatched during 1985–1992 ($n = 31$) and 1996–2002 ($n = 22$), respectively, whose known pedigree was sufficiently deep to estimate f . Songs were recorded using a WM-6DC Sony Professional Walkman and Sennheiser ME80 microphone (1988–1993) or an Optimus CTR-117 recorder and

Sennheiser ME67 microphone (2003), and analysed using a Multigon Uniscan II real-time analyser (songs recorded during 1988–1992) or the Syrinx sound analysis program (John Burt, www.syrinxpc.com, songs recorded in 1993 and 2003). We typed a minimum of 215 continuously recorded songs for each male; 210 songs are sufficient to estimate repertoire size with 95% confidence on Mandarte (Cassidy 1993). In most cases the number of songs analysed hugely exceeded this threshold (median 347, maximum 2558; 225 songs is sufficient to estimate repertoire size with 99% confidence, Cassidy 1993). In 1993 and 2003, we imposed an additional minimum criterion of 20 song bouts (exceeding the recommended minimum; Beecher *et al.* 2000). Each individual's repertoire size was therefore recorded with high confidence (see also Reid *et al.* 2004). Repertoire sizes were measured without knowledge of an individual's inbreeding level or immune response.

(c) Cell-mediated immunity

CMI is one major component of the avian acquired immune system (Wakelin 1996). We measured CMI in a total of 117 song sparrows during 16–24 February 2002 ($n=25$), 22 September–3 October 2002 ($n=47$) and 13–20 September 2003 ($n=45$) using a standard protocol: the wing-web (patagium) swelling response to subcutaneous injection of phytohaemagglutinin (PHA, Goto *et al.* 1978). Sparrows were mist-netted, biometrics were recorded and CMI was measured over a ca 18 h experimental period as described by Reid *et al.* (2003). A higher CMI score is taken to indicate a stronger immune response. Patagial measurements were highly repeatable ($r > 0.96$, $p < 0.0001$). Measurement error therefore introduced little uncertainty into CMI estimates (less than 5%; Reid *et al.* 2003). The 117 tested individuals included 19 males whose song repertoire size was recorded during 2003.

(d) Analyses

We used general linear mixed models to test whether a male's coefficient of inbreeding (f) explained a significant proportion of variation in song repertoire size. Preliminary analyses suggested that song sparrows recorded during 1988–1993 (hatched 1985–1992) sang smaller repertoires than those recorded during 2003 (hatched 1996–2002). We therefore included a male's natal year or natal period (1985–1992 versus 1996–2002) as random factors in these models. Although we did not expect repertoire size to vary with male age (Nordby *et al.* 2002), we tested for age effects by including a male's age at recording as a covariate. Since previous analyses suggested that phenotypes can vary with parental inbreeding coefficients in song sparrows (Reid *et al.* 2003), we also tested whether repertoire size varied with maternal or paternal f . However, parental inbreeding levels were not accurately known for most males recorded during 1988–1993 due to insufficient pedigree depth. We therefore restricted the analysis of parental effects to sparrows recorded during 2003.

We used further general linear models to test whether CMI varied with repertoire size or f . Preliminary analyses suggested that across all tested individuals, CMI differed between the three testing seasons ($n=117$, $F_{2,114}=4.6$, $p=0.01$) but not between males and females ($n=101$ individuals of known sex, $F_{1,99}=0.6$, $p=0.42$). We therefore standardized each male's CMI score (x) for testing season by

Figure 1. Relationship between a male song sparrow's coefficient of inbreeding (f) and song repertoire size for 31 males hatched during 1985–1992 (open symbols, dashed line) and 22 males hatched during 1996–2002 (filled symbols, solid line). Repertoire size differed between natal periods and declined with f ; more inbred males sang smaller repertoires ($n=53$, f : $F_{1,50}=15.9$, $p<0.001$, $\beta=20.5$; natal period: $F_{1,50}=7.5$, $p=0.009$; interaction: $p=0.16$). For clarity, markers are displaced with respect to repertoire size where multiple males had identical characteristics.

subtracting the mean CMI score (μ) for that season and dividing by the season-wide standard deviation (σ , thus $z=(x-\mu)/\sigma$; Zar 1999). We did not include 'testing season' as a random factor in models relating CMI to repertoire size and f because the 19 males for whom we measured both CMI and repertoire size included few that were tested during each season ($n=6$, 8 and 5, respectively). Since previous analyses suggested that CMI varied with age and body condition (Reid *et al.* 2003), we included these traits as covariates. Although we previously found no effect of parental f on CMI in fledged sparrows (Reid *et al.* 2003), we verified this result by including maternal and paternal f as covariates where possible. Individual ages were known because sparrows were ringed as chicks. Body condition was estimated as the residual of mass on the cube of the first principal component of wing and tarsus length. Sexes were attributed by wing length and breeding behaviour (Reid *et al.* 2003).

We used Spearman partial correlations to test whether correlations between inbreeding and repertoire size, inbreeding and CMI and CMI and repertoire size remained significant after controlling for variation in the third variable in each case.

Analyses were run in SPSS (v.10.0) and SAS (v.8.2). Covariates were eliminated from models if $p>0.1$. For eliminated variables, we present statistics associated with their reintroduction to the final model. Means are presented ± 1 s.e.

3. RESULTS

(a) Song repertoire size and inbreeding

Across 53 male song sparrows whose song repertoire sizes were recorded, repertoire size varied between 5 and 11 (8.0 ± 0.2) and f varied between 0.000 and 0.164 (0.034 ± 0.005). Repertoire size declined significantly with a male's coefficient of inbreeding ($n=53$, $F_{1,51}=8.0$, $p=0.007$). Support for this relationship increased after controlling for natal period (figure 1). Repertoire size did not differ

significantly among individual cohorts or vary with a male's age at recording (cohort: $F_{14,37}=1.7$, $p=0.10$; age: $F_{1,49}=0.2$, $p=0.65$). Across males hatched during 1996–2002, repertoire declined with individual f but not maternal f ($n=22$, f : $F_{1,19}=14.1$, $p=0.001$; maternal f : $F_{1,19}=1.5$, $p=0.23$) or paternal f ($n=18$, f : $F_{1,15}=9.2$, $p=0.008$; paternal f : $F_{1,15}=1.2$, $p=0.30$). Sample size was reduced in the latter analysis because paternal f was unknown for four offspring of immigrant males.

More inbred song sparrows may have sung smaller repertoires because inbreeding directly reduced a male's ability to learn, reproduce or innovate songs. However, we considered two mechanisms by which an indirect correlation between f and repertoire size could have arisen. First, the 53 recorded males included 12 offspring of immigrant breeders. If repertoire size has a genetic basis, these outbred individuals might sing larger repertoires due to heterosis resulting from between-population crosses (Falconer & Mackay 1996; Marr *et al.* 2002). Further, since songs differ between song sparrow populations (Cassidy 1993), immigrant males may sing different songs from Mandarte-born males on neighbouring territories. Outbred offspring of immigrant males might therefore be exposed to higher average song diversity early in life and learn larger repertoires as a consequence. However, the 12 offspring of immigrants did not sing larger repertoires than the seven outbred offspring of native Mandarte parents (means of 8.7 ± 0.5 and 8.4 ± 0.4 , respectively, $F_{1,17}=0.1$, $p=0.74$), and repertoire size still declined significantly with f when the 12 immigrants' offspring were excluded ($n=41$, f : $F_{1,38}=13.3$, $p=0.001$; natal period: $F_{1,38}=7.2$, $p=0.011$). We therefore rejected the hypothesis that repertoire size declined with f solely because outbred offspring of immigrant breeders had particularly large repertoires.

Second, our 53 males hatched in 15 different years, during which, population size and immigration rate varied. Close inbreeding is most likely in populations with a small effective size (N_e) and few immigrants (Falconer & Mackay 1996). Equally, small and isolated populations may show reduced song diversity if memes are lost by cultural drift and new memes are rarely introduced (Lynch & Baker 1994). We therefore investigated whether repertoire size declined with f because both traits varied independently with N_e or the proportion of adults that were immigrants in each male's natal year. We estimated N_e as $4N_mN_f/(N_m+N_f)$, where N_m and N_f equal the total number of adult males and females present in each year (Falconer & Mackay 1996). N_e was tightly correlated with N_f across years ($n=15$ years, $r=0.98$, $p<0.001$). As expected, f was higher when N_e (or N_f) was small and few immigrants bred in a male's natal year ($n=53$, year: $p>0.1$; N_e : $F_{1,50}=23.1$, $p<0.001$; immigrants: $F_{1,50}=13.1$, $p=0.001$). In contrast, repertoire size did not vary with N_e or the proportion of immigrants ($n=53$, year: $p>0.1$; N_e : $F_{1,51}=0.7$, $p=0.42$; immigrants: $F_{1,51}=0.1$, $p=0.80$), although a severe population crash in 1989 may partially explain why males hatched during the earlier recording period sang smaller repertoires on average (figure 1). The decline in repertoire size with f therefore remained significant when N_e and the proportion of immigrants were statistically controlled (partial

Figure 2. Relationship between a male song sparrow's cell-mediated immune response (CMI) and his song repertoire size. After standardizing for testing season, CMI increased significantly with a male's repertoire size and body condition (repertoire: $F_{1,16}=7.8$, $p=0.01$; body condition: $F_{1,16}=4.9$, $p=0.04$) but not age at testing ($F_{1,15}=0.1$, $p=0.74$).

correlation, $r_{49}=-0.33$, $n=53$, $p=0.02$). We therefore rejected the hypothesis that repertoire size declined with f solely because both traits varied with population size or immigration rate.

(b) Song repertoire size and cell-mediated immunity

Across 19 male song sparrows where we measured both CMI and repertoire size, standardized CMI increased significantly with repertoire size and body condition, but did not vary with the male's age at testing (figure 2).

(c) Cell-mediated immunity and inbreeding

Consistent with previous analyses of a larger dataset (Reid *et al.* 2003), CMI declined significantly with f across 19 males whose repertoire size was also recorded (figure 3). CMI also increased with body condition but did not vary with maternal f , paternal f or the male's age at testing (figure 3).

(d) Song repertoire size, inbreeding and immunity

Consistent with the pattern across all recorded males (figure 1), repertoire size declined significantly with f across the 19 males whose CMI was measured during 2002–2003 ($F_{1,17}=11.3$, $p=0.004$). Therefore, across these 19 males, repertoire size, CMI and f were all significantly correlated with each other. We tested whether the correlation between each pair of traits remained significant after statistically controlling for the third trait in each case. The inbreeding coefficient (f) predicted repertoire size and CMI after controlling for CMI and repertoire size, respectively, but the phenotypic correlation between CMI and repertoire size was no longer significant after controlling for f (figure 4).

4. DISCUSSION

(a) Song repertoire size and inbreeding

Song repertoire size is a classic example of a sexually selected ornamental trait, and has been suggested to indicate a male's genetic quality (Searcy 1992; Hasselquist *et al.* 1996). In song sparrows on Mandarte, repertoire size

Figure 3. Relationship between a song sparrow's coefficient of inbreeding (f) and cell-mediated immune response (CMI) across 19 males whose song repertoire size was also recorded. After standardizing for testing season, CMI declined significantly with f and increased with body condition (f : $F_{1,16}=6.5$, $p=0.02$; body condition: $F_{1,16}=3.5$, $p=0.08$). Effects of age and parental f were not significant (age: $F_{1,15}=0.2$, $p=0.68$; maternal f : $F_{1,15}=1.0$, $p=0.33$; paternal f : $F_{1,11}<0.1$, $p=0.90$). Sample size was reduced in the latter analysis because paternal f was unknown for four offspring of immigrant males.

declined with a male's coefficient of inbreeding and therefore indicated his relative heterozygosity. This correlation did not arise because outbred offspring of immigrant breeders had particularly large repertoires, or because repertoire size and inbreeding levels varied independently with population size or immigration rate. Therefore, while correlative data cannot prove causality, one plausible explanation for our results is that inbreeding reduced repertoire size directly. Although we did not investigate possible mechanisms, inbreeding effects are often manifested during development (Keller & Waller 2002), and early developmental and nutritional stress can permanently affect brain development and song performance in *Melospiza* sparrows and other passerines (Nowicki *et al.* 2002; Buchanan *et al.* 2003). Repertoire size may therefore be a reliable indicator of inbreeding because inbreeding reduces neurogenesis or brain capacity; inbreeding effects on brain function are known from rats (*Rattus norvegicus*; Harker & Whishaw 2002) and humans (*Homo sapiens*; Bashi 1977).

Irrespective of its mechanistic basis, the inbreeding depression that we observed in song repertoire size is likely to be biologically meaningful. Inbreeding explained 24% of variation in the expression of this sexually selected trait. Female song sparrows choosing males by their repertoire size would on average acquire more outbred and therefore more heterozygous mates. Since a song sparrow's repertoire comprises relatively few song types, reduction by a single type represents a large proportional change. Repertoire size is therefore likely to be a relatively insensitive indicator of male 'quality' compared to ornamental traits where smaller adjustments are possible (e.g. tail length). Nevertheless, a relatively low inbreeding level ($f \approx 0.0625$, equivalent to a first-cousin pairing) was associated with a reduction of *ca* one whole song type and would therefore be detectable. Ornamentation has previously been shown to decline with close inbreeding in laboratory populations. For example, inbred lines ($f \geq 0.25$) showed reduced male courtship song in

Figure 4. Spearman partial correlations between standardized cell-mediated immunity (CMI) and song repertoire size controlling for inbreeding ($r_s(f)$), inbreeding and repertoire size controlling for CMI ($r_s(\text{CMI})$) and inbreeding and CMI controlling for repertoire size ($r_s(\text{song})$).

Drosophila subobscura and *Drosophila montana* (Maynard Smith 1956; Aspi 2000) and ornamental colouration in guppies (*Poecilia reticulata*; van Oosterhout *et al.* 2003). Our results complement these studies by showing that a sexually selected ornamental trait can indicate inbreeding levels occurring among free-living individuals.

Effects of inbreeding and heterozygosity on ornamentation may not be unusual. The inbreeding levels that we observed may occur commonly in natural populations of song sparrows and other species (Keller & Waller 2002; Keller *et al.* 2005). Furthermore, recent studies have showed that ornamentation can decline with measures of local heterozygosity (e.g. multi-locus heterozygosity, Foerster *et al.* 2003; mean d^2 , Marshall *et al.* 2003). Although these measures are likely to be weak estimators of inbreeding and genome-wide heterozygosity (Slate *et al.* 2004), the implication is that ornamentation may reflect heterozygosity at specific loci and, therefore, genetic dominance effects.

The observation that secondary sexual ornaments can reflect genetic dominance effects has implications for the broader understanding of evolutionary mechanisms of intersexual selection (Charlesworth 1988). First, genetic dominance effects influence the response of any system to selection, and therefore the course of evolution (Falconer & Mackay 1996). Specifically, patterns of evolution can vary with population size and structure when genetic dominance effects are present (Whitlock 2003). However, many genetic models of sexual selection and female choice focus on haploid genetic systems, and thereby ignore dominance effects by definition (Andersson 1994). Explicit consideration of these effects may clarify links between trajectories of sexual selection and population structure, issues that are pertinent to speciation models and conservation strategies for sexually selected species (Kokko & Brooks 2003; Kirkpatrick & Ravigné 2002). Second, in species such as song sparrows where males contribute to parental care, female preferences for outbred males may have evolved primarily for direct fitness benefits. However, because parental heterozygosity can increase offspring heterozygosity (Mitton *et al.* 1993), choice for outbred males may evolve for purely indirect genetic benefits, at least under some conditions (Charlesworth 1988; Irwin & Taylor 2000; Reinhold 2002). Particularly with respect to such systems, the extent to which female choice for heterozygous males might directly maintain genetic variance in systems

under sexual selection deserves further theoretical consideration.

(b) *Song repertoire size and immunity*

Relationships between ornamentation and male fitness cannot be reliably described by measuring single fitness components, such as immunity, because individual components may be negatively correlated with each other and with overall fitness (Kokko *et al.* 2002, 2003). However, relationships between ornamentation and immunity are of specific interest because host–parasite interactions have been hypothesized to maintain additive genetic variance in traits under sexual selection (Hamilton & Zuk 1982; Andersson 1994; Westneat & Birkhead 1998). Numerous pathways linking ornamentation to immunity have been proposed (Folstad & Karter 1992; Westneat & Birkhead 1998; Kurtz & Sauer 1999), and phenotypic correlations between ornamentation and immunity have been described (Møller *et al.* 1999). Yet few studies have identified genetic mechanisms that underlie these phenotypic relationships.

Consistent with studies relating song to immunity in other species (Ryder & Siva-Jothy 2000; Duffy & Ball 2002; Buchanan *et al.* 2003), song repertoire size increased significantly with CMI in song sparrows. Therefore, female song sparrows choosing males by repertoire size would on average acquire mates with greater phenotypic immunity. However, the phenotypic correlation that we observed between repertoire size and CMI was no longer significant after correlated effects of inbreeding were statistically controlled. Since this analysis was based on 19 males, we cannot exclude the possibility that a weak causal link between CMI and repertoire size remained undetected due to insufficient statistical power. However, this possibility does not alter our conclusion that the significant positive correlation that we initially observed between these traits could be explained by covariation with inbreeding. Rather than indicating immunity directly, a male song sparrow's repertoire size primarily indicated his inbreeding level, a non-additive genetic predictor of his response to an experimental immune challenge.

In the most general terms, the patterns that we observed are consistent with Hamilton & Zuk's (1982) hypothesis that ornamentation indicates the genetic basis of immunity. However, this basis comprised heterozygosity and therefore differed from the additive 'good genes' envisaged in models of parasite-mediated sexual selection (Hamilton & Zuk 1982; Andersson 1994). Since genetic dominance contributes to additive genetic variance (Falconer & Mackay 1996) our data do not imply an absence of additive variance in repertoire size or immunity. Nor can we exclude the possibility that repertoire size indicates additive genetic variance in components of immunity that we did not study. Our results should not, therefore, be interpreted as evidence for or against Hamilton and Zuk's hypothesis. However, our results do suggest that significant positive phenotypic correlations between ornamentation and immunity may be attributable to a non-additive genetic mechanism. More caution may therefore be warranted when interpreting such phenotypic correlations as evidence for Hamilton and Zuk's additive theory of parasite-mediated sexual selection.

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