Molecular Ecology (2003) **12**, 3077–3083 doi: 10.1046/j.1365-294X.2003.01968.x

Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird

COREY R. FREEMAN-GALLANT,* MICHAEL MEGUERDICHIAN,* NATHANIEL T. WHEELWRIGHT† and SUZANNE V. SOLLECITO*

**Department of Biology, Skidmore College, Saratoga Springs, New York, USA, 12866, †Department of Biology, Bowdoin College, Brunswick, Maine, USA, 04011*

Abstract

Female birds often copulate outside the pair-bond to produce broods of mixed paternity, but despite much recent attention the adaptive significance of this behaviour remains elusive. Although several studies support the idea that extra-pair copulations (EPCs) allow females to obtain 'good genes' for their offspring, many others have found no relationship between female mating fidelity and traits likely to reflect male quality. A corollary to the good genes hypothesis proposes that females do use EPCs to increase the quality of young, but it is the interaction between maternal and paternal genomes — and not male quality *per se* — that is the target of female choice. We tested this 'genetic compatibility' hypothesis in a free-living population of Savannah sparrows (*Passerculus sandwichensis*) by determining whether females mated nonrandomly with respect to the major histocompatibility complex (*Mhc*). During both the 1994 and 1995 breeding seasons, female yearlings (but not older birds) avoided pairing with *Mhc*-similar males ($P < 0.005$). The *Mhc* similarity between mates also predicted the occurrence of extra-pair young in first broods ($P < 0.007$) and covaried with estimates of genome-wide levels of similarity derived from multilocus DNA fingerprinting profiles ($P = 0.007$). The overall genetic similarity between adults tended to predict female mating fidelity, but with less precision than their *Mhc* similarity ($P = 0.09$). In contrast, females appeared insensitive to the size, weight or age of males, none of which explained variation in female mating fidelity. Taken together, these results are consistent with the hypothesis that females sought complementary genes for their offspring and suggest either that the benefits of heterozygosity (at the *Mhc*) drive female mating patterns or that the avoidance of inbreeding is an ultimate cause of social and genetic mate choice in Savannah sparrows.

Keywords: extra-pair paternity, good genes, individual optimization, Savannah sparrow, sexual selection

Received 30 June 2003; revision received 17 July 2003; accepted 17 July 2003

Introduction

After more than a decade of applying DNA fingerprinting techniques to free-living populations of birds, it now seems clear that both males and females readily copulate outside the pair-bond and that broods of mixed paternity are common in even socially monogamous species (Birkhead 1998; Hasselquist & Sherman 2001). Less well understood is why females engage in this genetic polyandry. While extra-pair males can increase their reproductive success by parasitizing the parental care of the individuals they cuckold, females incur numerous costs and few obvious benefits by mating outside of the pair-bond. Female infidelity has been linked to lower nest defence (Weatherhead *et al*. 1994) and feeding rates (Dixon *et al*. 1994) by cuckolded males, and infidelity may increase a female's exposure to disease and predation (Westneat *et al*. 1990). None the less, females

Correspondence: Corey R. Freeman-Gallant. Fax: (518) 580 5071; E-mail: cfreeman@skidmore.edu

often appear to pursue EPCs actively, suggesting that they benefit from mating multiply (Kempenaers *et al*. 1992; Lifjeld & Robertson 1992; Hasselquist *et al*. 1995; Strohbach *et al*. 1998).

Because females seem to obtain only sperm (and fertilizations) from extra-pair males, much recent attention has focused on indirect, genetic benefits of extra-pair paternity (Birkhead & Møller 1992; Birkhead 1998; but see Gray 1997). According to the 'good genes' hypothesis, females paired with suboptimal males can improve the quality of their young by securing fertilizations from higher quality mates. This hypothesis is not without support, and several intraspecific studies have now shown that females allocate paternity to males with high survivorship, as revealed by the size (Kempenaers *et al*. 1992; Weatherhead & Boag 1995), song (Hasselquist *et al*. 1996), age (Richardson & Burke 1999) or plumage (Sheldon *et al*. 1997) of extra-pair sires.

Although the good genes hypothesis generally predicts concordant patterns of female choice — all females in a given population should prefer the same, high quality males — individual optimization is not inconsistent with the good genes scenario (Brown 1997). For example, females might mate so as to minimize the risk of genetic incompatibility between maternal and paternal genomes (Zeh & Zeh 1996, Zeh & Zeh 1997), avoid the costs of inbreeding (Blomqvist *et al*. 2002) or optimize offspring heterozygosity at one or several key loci (Jennions & Petrie 2000; Tregenza & Wedell 2000). Because the set of best (or most complementary) males will depend on a female's genotype, only genetically similar females should agree on male quality.

A probable target for individual optimization is the major histocompatibility complex (*Mhc*), an important component of acquired immunity in vertebrates. The products encoded by class I and class II genes present antigens to helper and cytotoxic T cells, thereby eliciting the adaptive immune response to infection (Edwards & Potts 1996; Wakelin & Apanius 1997). The *Mhc* may thus fit well with traditional good genes models of female choice: by prefering relatively disease-resistant males, female may be securing 'good' *Mhc* haplotypes for their young (von Schantz *et al*. 1996; Paterson *et al*. 1998; Ditchkoff *et al*. 2001; Zelano & Edwards 2002). More generally, however, immunocompetence may not be conferred by the presence or absence of especially fit haplotypes, but instead by the *combination* of maternal and paternal genes. If heterozygotes are able to mount an immune response against a broader array of pathogens than homozygotes, females should mate disassortatively in order to increase the quality of young (Brown 1997; Jennion & Petrie 2000; Tregenza & Wedell 2000).

In birds, disassortative mating can occur through nonrandom pairing or, separately, through cuckoldry and the choice of appropriate extra-pair sires. In bluethroats (*Luscinia svecica*), extra-pair fertilizations result in offspring with greater immunocompetence than both their maternal and paternal half-sibs, implying that particular combinations of genes, obtained via EPCs, improve offspring quality (Johnsen *et al*. 2000). However, no study has yet determined whether female birds prefer genetically dissimilar males, as now appears to be the case in other vertebrates (for example, Potts *et al*. 1991; Landry *et al*. 2001).

We tested for the presence of disassortative mating in a known-age, marked population of Savannah sparrows (*Passerculus sandwichensis*) breeding on Kent Island, New Brunswick, Canada in 1994 and 1995. As many as 60% of female Savannah sparrows produce young outside of the pair-bond (Freeman-Gallant 1997), and intraseasonal changes in female mating fidelity have already been linked to indices of male quality (Freeman-Gallant 1996). However, the determinants of first-brood mating fidelity have not been fully explored.

Methods

Field protocols

The behavioural ecology of Savannah sparrows has been studied intensively on Kent Island (44°35′ N, 66°46′ W) since 1987 (Freeman-Gallant 1998; Wheelwright & Mauck 1998). Beginning in mid-May each year, we captured all unbanded adults nesting in our study site with mist-nets and fitted each bird with a USFWS aluminium band and a unique combination of three-coloured leg bands. At the time of banding, we measured unflattened wing chord and obtained the mass of each adult. We also confirmed the gender of each bird based on the presence or absence of a cloacal protuberance (male) or brood patch (female) and took a small (50–75 µL) sample of blood from the brachial vein. Blood was stored in a lysis buffer at –20 °C. Offspring were banded and a blood sample taken 7 days after hatching. Only first-brood pairings (and young) are considered in the present study.

Mhc *similarity and social pairing*

To assess the importance of *Mhc* genotype to social and genetic mating patterns, we generated restriction fragment length polymorphism (RFLP) profiles for each adult using a 209-base pair (bp) fragment of a class II β gene cloned from Savannah sparrows (Freeman-Gallant *et al*. 2002). Whole genomic DNA was isolated from lysed blood using a series of phenol–chloroform extractions and digested to completion with *Eco*RI. We electrophoresed digest products on a 0.8% agarose gel for 1568 volt-hours and transferred DNA to a nylon membrane. After hybridization to the *Mhc* probe, RFLP patterns were detected under high stringency with Roche's nonradioactive (Genius) system and transferred manually to an acetate sheet by marking each band in its centre. The marked sheet was then scanned into Adobe

PhotoShop and the size of each band determined according to its position relative to molecular weight markers. The presence of several fixed bands facilitated the comparison of RFLP profiles across gels, allowing us to construct an unambiguous catalogue of *Mhc* genotypes for birds breeding in each of the 2 years.

We used the fraction of DNA restriction fragments shared between birds as a crude estimate of underlying sequence similarity at the *Mhc* (after Nei & Li 1979). *Mhc* similarity was calculated as twice the number of RFLP bands shared between any two birds x and y divided by the total number of bands scored in the two individuals. Unlike mammals, but like some birds, segregation analysis of RFLP profiles suggests that the *Mhc* is organized into two separate gene clusters in Savannah sparrows, each containing at least several class II genes (Briles *et al*. 1993; Wittzell *et al*. 1995; Freeman-Gallant *et al*. 2002). Band-sharing at the *Mhc* is likely to reflect the number of haplotypes shared between males and females (0–4 haplotypes) as well as the 'background' similarity among the haplotypes themselves (different haplotypes may have restriction sites in common and therefore share some RFLP bands). However, we cannot exclude the possiblility that some bands represent the presence of pseudogenes or gene fragments outside the sparrow's *Mhc*. As a consequence, band-sharing at the *Mhc* could also reflect overall levels of genetic similarity between adults, akin to the measures of similarity derived from multilocus DNA fingerprinting profiles (for example, see Blomqvist *et al*. 2002).

We calculated the proportion of RFLP bands shared between each female and her social mate as well as between each female and 34–36 other males breeding in the population at the same time. This allowed us to rank a female's similarity to her social mate against her similarity to other potential sires in the population. In 1994, females were compared to 35 males (83.3% of all males in the study site); in 1995, females were compared to 37 males (representing 71.2% of all locally available mates). Pooling over the 2 years, the average *Mhc* band-sharing between females and males was 0.74 ± 0.11 (SD) and ranged from 0.27 to 1.00.

Using Monte Carlo methods (Manley 1991), we compared the observed number of *Mhc*-similar matings to the distribution of such matings obtained by randomly pairing males and females over 10 000 iterations. We considered as '*Mhc*-similar' a mating between a female and male ranked in the first third of the distribution of all males for that female (i.e. males ranked 1–12). We examined two different null models, one in which males were assigned to females with replacement (so that every male was available to all females and polygynous pairing might occur) and one without replacement (to incorporate constraints on social mate choice imposed by prior settling females). Because the results of both models were similar, we report only the results of the latter model here.

We focused our modelling on the number of *Mhc*-similar pairings rather than on the number of *Mhc*-dissimilar pairings because it is unclear whether females should prefer males showing intermediate or the lowest levels of similarity. Under some circumstances, choosy females might be expected to avoid the most *Mhc*-dissimilar males: for example, if extreme heterozygosity has the effect of decreasing immunocompetence (Penn & Potts 1999; Reusch *et al*. 2001) or, more generally, if outbreeding depression occurs on Kent Island and the *Mhc* is used as a genetic marker of relatedness (Brown & Eklund 1994).

Female mating fidelity

We screened for the presence of extra-pair offspring using *Hae*III and an end-labelled oligonucleotide [$(GGAT)_4$] probe to generate multilocus DNA fingerprinting profiles (after Chuang *et al*. 1999). Exclusion criteria were based on the proportion of bands shared between offspring and putative parents and the presence of novel fragments in young. Among 58 (within-pair) offspring with no more than a single novel fragment, only two shared fewer than 50% of their bands with their mother (assumed to be the true mother because intraspecific brood parasitism is rare or absent in this population; Freeman-Gallant 1996). Thirteen of these 58 offspring had one novel fragment, suggesting the occurrence of three novel fragments by chance mutation or scoring error alone was less than 2%. Thus, offspring were assumed to derive from fertilizations outside of the pair bond if they shared fewer than 50% of their bands with their putative father and possessed three or more novel fragments.

For 95 of 116 offspring, the two exclusion criteria categorized young similarly. We used an additional restriction enzyme (*Alu*I) to confirm extra-pair ($N = 8$) or within-pair ($N = 1$) status for some offspring that met or exceeded only one of the two criteria. Of the remaining 12 ambiguous cases, 11 were in nests with extra-pair young [so that assignment of female mating status (faithful vs. unfaithful) was unaffected by the ambiguity]. One offspring with only two novel fragments and a marginally low band-sharing with its putative father (48%) was assumed to be within-pair.

We determined the status of an additional 29 offspring during segregation analysis of *Mhc* bands (Freeman-Gallant *et al*. 2002). Young were assumed to derive from extra-pair fertilizations if they possessed *Mhc* fragments found in neither putative parent. Where more than one extra-pair offspring was present in a brood, the same novel fragments tended to appear in multiple young, suggesting that mutation cannot explain the occurrence of the novel bands.

Female age and sample size

Because Kent Island Savannah sparrows show strong natal and breeding philopatry (Wheelwright & Mauck 1998), we were able to study birds of known age. Pooling over 1994 and 1995, we examined social mating patterns among 46 females of known age, divided equally into yearling and after-second-year age classes. We examined the mating fidelity of 32 of these birds, along with an additional 11 females whose age class was unknown.

Because individuals can be classified as yearlings only once in their lifetime, analyses that examine disassortative mating among second-year females cannot be confounded by pseudoreplication. However, four older females that nested on Kent Island in 1994 returned to breed in 1995. Following these four females longitudinally, one renested with the same male, one chose a less *Mhc*-similar male, one chose a more *Mhc*-similar male and one paired with a male almost identical to her original social mate. Systematically excluding these four females from either year does not change the *Mhc*-random pattern observed among older birds. The summary statistics described below therefore include all four females in both years.

Among the 43 females included in analyses exploring the relationship between *Mhc* similarity and occurrence of extra-pair paternity, five females appeared in both 1994 and 1995. Because four of these five females paired with different males in the 2 years, we treat each pairing as an independent event. However, if females are allowed to appear only once in each analysis (by randomly excluding data from 1 of the 2 years) the results are unchanged. The statistics reported below therefore include data on all birds in each year.

Results and discussion

Yearling Savannah sparrows did not pair at random with respect to RFLP variation at the *Mhc*. Pooling over the 2 years, only two of 23 yearling females (8.7%) paired with *Mhc*-similar males despite the fact that such males were readily available in the population (Fig. 1; Monte Carlo, $P < 0.005$). In contrast, older females paired at random with respect to the *Mhc*; nine of 23 older females paired with similar males (Monte Carlo, $P > 0.50$). As a consequence of disassortative pairing by yearling females but not older birds, the average *Mhc* similarity rank was lower between yearling females and their social mates than between older females and their social mates (two-way ANOVA with year as a covariate, $F_{1,42} = 4.5$, $P = 0.04$). Absolute *Mhc*-bandsharing was lower between yearling females and their social mates as well (two-way ANOVA, $F_{1,42} = 4.7$, $P = 0.04$).

The difference in social mating patterns between female age groups might reflect constraints imposed by extreme breeding philopatry (Wheelwright & Mauck 1998). Although none of the 23 older females moved more than 150 m between nesting attempts in consecutive years, only three females paired again with the same male, mainly because their original partners had died (16 of 20 females). In some cases, *Mhc*-dissimilar males may not be available to returning females in the immediate vicinity of the females' previous nests.

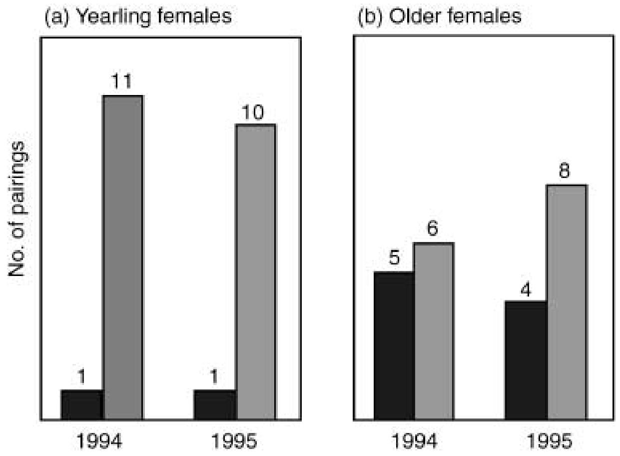

Fig. 1 Observed number of social pairings between females and *Mhc*-similar males (males ranked 1–12; black boxes) and between females and more *Mhc*-dissimilar males (males ranked 13–35; grey boxes). Yearling females (*a*) but not older birds (*b*) tended to avoid *Mhc*-similar males in both 1994 and 1995.

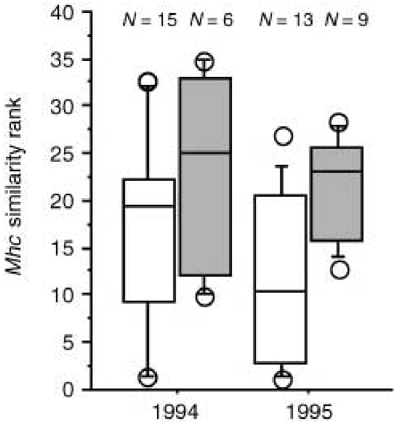

Fig. 2 In both 1994 and 1995, females producing extra-pair young (open boxes) were mated to more *Mhc*-similar males than females producing only within-pair young (shaded boxes). High ranks (e.g. ranks 1–12) indicate a high degree of *Mhc* similarity. Boxes show the interquartile range, median (central hatch), 10th and 90th percentiles (whiskers) and presence of outliers (points).

Social mate choice is not the only avenue through which female Savannah sparrows can mate disassortatively, however. Pooling over 1994 and 1995, extra-pair offspring occurred in 28 of 43 nests (65.1%) and 69 of 145 offspring (47.6%) derived from fertilizations outside of the pair-bond. The occurrence of extra-pair paternity was predicted strongly by a female's similarity to her social mate. In both years, females producing only within-pair young were paired to more *Mhc*-dissimilar males than females producing at least one extra-pair offspring (Fig. 2: $F_{1,39} = 8.26$, $P = 0.0065$). In a logistic regression that used similarity rank to predict the presence or absence of extra-pair offspring in a female's brood (and included year and female age as covariates), only *Mhc* similarity was significant (Wald $\chi^2 = 10.0$, $P = 0.0065$). In contrast, the occurrence of extra-pair offspring in Savannah sparrows was not predicted by a male's size, weight, or age, regardless of whether these variables were considered singly or in combination with the male's *Mhc* similarity rank (logistic regressions, $P > 0.30$).

The inability of male phenotypic traits to predict female mating fidelity is expected under individual optimization models of female choice. If 'good genes' are complementary genes, only females possessing similar genotypes should exhibit concordant mating preferences. Such individual optimization (at the *Mhc* or other loci) could help to explain the growing number of studies in which female birds appear to mate at random with respect to the morphology and behaviour of males (e.g. Dunn *et al*. 1994; Hill *et al*. 1994; Stutchbury *et al*. 1997; Strohbach *et al*. 1998; Webster *et al*. 2001).

We emphasize that individual optimization and traditional good genes explanations of female choice are not necessarily alternatives, however. Females might choose complementary males among the subset of males with the best genes or, vice versa, might select the most vigorous or elaborate males among those showing similar levels of compatibility. The criteria of compatibility and overall genetic quality can also be applied simultaneously or sequentially by females, depending on breeding phenology. In Savannah sparrows, we have shown previously that females alter their mating fidelity between broods according to the amount of paternal care directed towards first-brood young, a reasonable predictor of a male's over-winter survivorship (Freeman-Gallant 1996). We speculate that females might base first-brood mating fidelity on genetic similarity but then update their assessment of overall male quality based on their experience with male parental effort. Whether females are ever forced to trade overall male quality against compatibility is unknown, but must depend on the genetic characteristics of the males available as extra-pair sires.

Interestingly, only *Mhc* similarity rank and not band-sharing at the *Mhc per se* predicted female fidelity on Kent Island (multiple logistic regression with year, female age and both similarity indices as covariates, effect of *Mhc* band-sharing: *Wald* $\chi^2 = 1.46$, $P = 0.23$; effect of similarity rank: *Wald* $\chi^2 = 6.65$, $P = 0.01$). Thus, a female showing high *Mhc* band-sharing with her social partner tended to mate outside the pair-bond only if more dissimilar males were readily available in the population (so that the social male had a high *Mhc* similarity ranking). This result suggests the presence of an open-ended preference for relatively dissimilar males and not the targeting of particular or threshold levels of *Mhc* similarity.

Although we have no direct experimental evidence demonstrating a causal link between *Mhc* similarity and the sparrows' mating patterns, our correlative data suggest that females can discriminate between males carrying different *Mhc* haplotypes. Both mammals (reviewed in Penn & Potts 1999) and fish (Reusch *et al*. 2001) appear to use odour cues, and it seems possible that birds might also detect quantitative differences in their similarity to potential sires via olfaction. The olfactory acuity of passerines is comparable to the acuity of some mammals despite the relatively small size of their olfactory bulbs (Clark *et al*. 1993), and odours seem to be used during courtship in at least some avian taxa (Hagelin *et al*. 2002).

More generally, it seems surprising that our estimate of *Mhc* similarity is predictive. In salmon (*Salmo salar*), only amino-acid similarity at the immunologically important, peptide-bind region (PBR) predicts female mating patterns (Landry *et al*. 2001), and although our class II *Mhc* probe also targets the PBR in sparrows (Freeman-Gallant *et al*. 2002), similar RFLP profiles are likely to reflect the presence of identical restriction sites (and underlying sequence similarity) more broadly across the *Mhc*. Similar profiles could miss functionally different PBRs while, conversely, different RFLP haplotypes might be immunologically equivalent. None the less, *Mhc* genotypes detected by a similar approach in ring-necked pheasants (*Phasianus colchicus*) were also linked to mating success (von Schantz *et al*. 1996), suggesting that even RFLP variation can be informative.

Importantly, nonrandom patterns of social pairing and extra-pair paternity at the *Mhc* do not necessarily imply that the *Mhc* itself is the proximate target of female choice. For example, any inbreeding avoidance mechanism that prevents fertilizations between close relatives could generate the appearance of disassortative mating at the *Mhc* because matings between genetically similar birds would be precluded. Such a scenario is unlikely to account for the patterns observed in Savannah sparrows, however. We have pedigree information for 18 of the 23 yearling females included in this study, and only seven females had close kin (a putative father or brother) among the 35–37 males to which they were compared. Moreover, in each of these seven cases, only a single relative was available as a potential mate. A wholesale avoidance of these few kin cannot account for the broader patterns described here.

We cannot exclude the possibility that our results reflect the avoidance of more distantly related kin. Although our segregation analysis of RFLP bands in Savannah sparrows failed to reveal the presence of more than two independently assorting *Mhc* loci (Freeman-Gallant *et al*. 2002), it is possible that our probe detects class II gene fragments and/or pseudogenes dispersed throughout the sparrow genome. If true, our similarity indices may conflate similarity at the *Mhc per se* with genome-wide levels of similarity

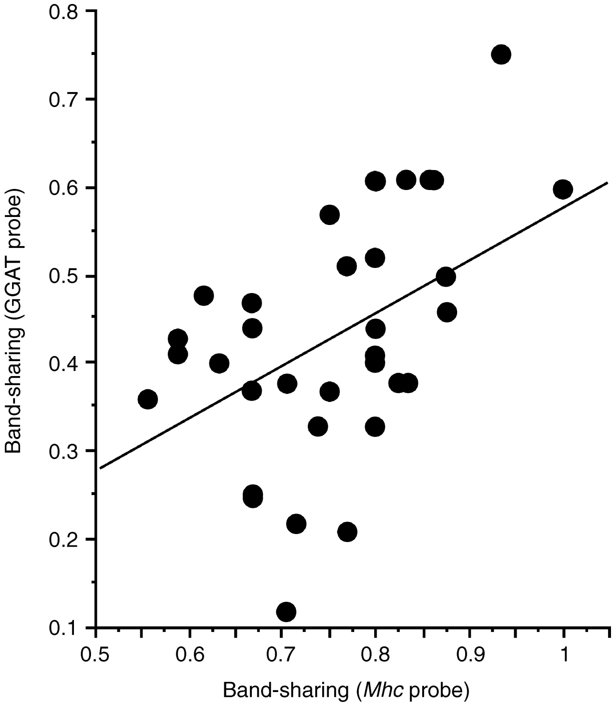

Fig. 3 Relationship between absolute *Mhc* similarity (band-sharing) and genome-wide levels of similarity derived from multilocus DNA fingerprinting profiles [$(GGAT)_4$ probe] for 33 female Savannah sparrows and their social mates.

and relatedness. This limitation associated with our methodology is particularly problematic (and interesting) because the *Mhc* itself can act as a genetic marker of relatedness (Brown & Eklund 1994; Penn & Potts 1999) which, in turn, may influence the occurrence of extra-pair paternity in birds (Blomqvist *et al*. 2002) and mammals (Potts *et al*. 1991).

Among Savannah sparrows, absolute *Mhc* band-sharing was a good predictor of the overall genetic similarity between females and their social mates (Fig. 3: $R^2 = 0.19$, $P = 0.007$), as estimated by quantifying the proportion of $(GGAT)_4$ fingerprinting bands shared between adults (after Bensch *et al*. 1994; Kempanaers *et al*. 1996; Blomqvist *et al*. 2002). Moreover, the probability that females produced extra-pair young tended to increase with increasing levels of genetic similarity (logistic regression with year as a covariate, Wald $\chi^2 = 2.9$, $P = 0.09$) among the subset of females for which we had $(GGAT)_4$ data ($N = 33$). Whether disassortative mating at the *Mhc* is the proximate mechanism underlying this pattern or whether the broader *Mhc* results simply reflect the avoidance of genetically similar (but putatively unrelated) individuals is not known. Regardless, these data strongly suggest an important role for individual optimization and genetic compatibility in explaining female mating fidelity in songbirds.

Acknowledgements

We thank A. Freeman-Gallant for the Monte Carlo simulations and M. Palmer, S. Weirick, W. Markham, K. O'Connor, E. Johnson, F. Saponara, R. Schwab and K. Lamb for assistance in the field or laboratory. The manuscript was improved greatly by the comments provided by three anonymous referees. This work was supported by the National Science Foundation (IBN-0116139).

References

Bensch S, Hasselquist D, von Schantz T (1994) Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler? *Evolution*, **48**, 317–326.

Birkhead TR (1998) In: *Sperm Competition and Sexual Selection* (eds Birkhead TR, Møller AP), pp. 579–622. Academic Press, London.

Birkhead TR, Møller AP (1992) *Sperm Competition in Birds: Evolutionary Causes and Consequences*. Academic Press, London.

Blomqvist D, Andersson M, Küpper C *et al.* (2002) Genetic similarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **419**, 613–615.

Briles WE, Goto RM, Auffrey C, Miller MM (1993) A polymorphic system related to but genetically independent of the chicken major histocompatibility complex. *Immunogenetics*, **37**, 408–414.

Brown JL (1997) A theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8**, 60–65.

Brown JL, Eklund A (1994) Kin recognition and the major histocompatibility complex: an integrative review. *American Naturalist*, **143**, 435–461.

Chuang HC, Webster MS, Holmes RT (1999) Extra-pair paternity and local synchrony in the black-throated blue warlber. *Auk*, **116**, 726–736.

Clark L, Avilova KV, Bean NJ (1993) Odor thresholds in passerines. *Comparative Biochemistry and Physiology*, **104**, 305–312.

Ditchkoff SS, Lochmiller RL, Masters RE, Hoofer SR, Van Den Bussche RA (2001) Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (*Odocoileus virginianus*): evidence for good-genes advertisement. *Evolution*, **55**, 616–625.

Dixon A, Ross D, O'Malley SLC, Burke T (1994) Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. *Nature*, **371**, 698–700.

Dunn PO, Robertson RJ, Michaud-Freeman D, Boag PT (1994) Extra-pair paternity in tree swallows: why do females mate with more than one male? *Behavioral Ecology and Sociobiology*, **35**, 273–281.

Edwards SV, Potts WK (1996) In: *Molecular Genetic Approaches in Conservation* (eds Smith TB, Wayne RK), pp. 214–237. Oxford University, New York.

Freeman-Gallant CR (1996) DNA fingerprinting reveals female preference for male parental care in Savannah sparrows. *Proceedings of the Royal Society, London Series B*, **263**, 157–160.

Freeman-Gallant CR (1997) Extra-pair paternity in monogamous and polygynous Savannah sparrows, *Passerculus sandwichensis*. *Animal Behaviour*, **53**, 397–404.

Freeman-Gallant CR (1998) Fitness consequences of male parental care in Savannah sparrows. *Behavioral Ecology*, **9**, 486–492.

Freeman-Gallant CR, Johnson EM, Saponara F, Stanger M (2002) Variation at the MHC in Savannah sparrows. *Molecular Ecology*, **11**, 1125–1130.

Gray EM (1997) Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Animal Behavior*, **53**, 625–639.

Hagelin JC, Jones IL, Rasmussen LEL (2002) A tangerine-scented pheromone in a monogamous seabird [Abstract 182]. *North American Ornithological Congress*. 24–28 September 2002, New Orleans, LA.

Hasselquist D, Bensch S, von Schantz T (1995) Low frequency of extrapair paternity in the polygynous great reed warbler, *Acrocephalus arundinaceus*. *Behavioral Ecology*, **6**, 27–38.

Hasselquist D, Bensch S, von Schantz T (1996) Correlation between male song repertoire, extra-pair paternity, and offspring survival in the great reed warbler. *Nature*, **381**, 229–232.

Hasselquist D, Sherman PW (2001) Social mating systems and extrapair fertilizations in passerine birds. *Behavioral Ecology*, **12**, 457–466.

Hill GE, Montgomerie R, Roeder C, Boag PT (1994) Sexual selection and cuckoldry in a monogamous songbird: implications for sexual selection theory. *Behavioral Ecology and Sociobiology*, **35**, 193–199.

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, **75**, 21–64.

Johnsen A, Andersen V, Sunding C, Lifjeld JT (2000) Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature*, **406**, 296–298.

Kempenaers B, Adriaensen F, van Noordwijk AJ, Dhondt AA (1996) Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proceedings of the Royal Society London, Series B*, **263**, 179–185.

Kempenaers B, Verheyen GR, van den Broeck M, Burkey T, van Vroeckhoven C, Dhondt AA (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature*, **357**, 494–496.

Landry C, Garant D, Duchesne P, Bernatchez L (2001) 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society London, Series B*, **268**, 1279–1285.

Lifjeld JT, Robertson RJ (1992) Female control of extra-pair fertilizations in tree swallows. *Behavioral Ecology and Sociobiology*, **31**, 89–96.

Manley B (1991) *Randomization and Monte Carlo Methods in Biology*. Chapman & Hall, New York.

Nei M, Li W-H (1979) Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA*, **76**, 5269–5273.

Paterson S, Wilson K, Pemberton JM (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*). *Proceedings of the National Academy of Sciences USA*, **95**, 3714–3719.

Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. *American Naturalist*, **153**, 145–164.

Potts WK, Manning CJ, Wakeland EK (1991) Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, **352**, 619–621.

Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414**, 300–301.

Richardson DS, Burke T (1999) Extra-pair paternity in relation to male age in Bullock's oriole. *Molecular Ecology*, **8**, 2115–2126.

von Schantz T, Wittzell H, Göransson G, Grahn M, Persson K (1996) *MHC* genotype and male ornamentation: genetic evidence for the Hamilton–Zuk model. *Proceedings of the Royal Society London, Series B*, **263**, 265–271.

Sheldon BC, Merilä J, Qvarnström A, Gustafsson L. Ellegren H (1997) Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proceedings of the Royal Society London, Series B*, **264**, 297–302.

Strohbach S, Curio E, Bathen A, Epplen JT, Lubjuhn T (1998) Extra-pair paternity in the great tit (*Parus major*): a test of the 'good genes' hypothesis. *Behavioral Ecology*, **9**, 388–396.

Stutchbury BJ, Piper WH, Neudorf DL *et al.* (1997) Correlates of extra-pair fertilization success in hooded warblers. *Behavioral Ecology and Sociology*, **40**, 119–126.

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013–1027.

Wakelin D, Apanius V (1997) In: *Host–Parasite Evolution: General Principles and Avian Models* (eds Clayton DH, Moore J), pp. 30–58. Oxford University, New York.

Weatherhead PJ, Montgomerie R, Gibbs HL, Boag PT (1994) The cost of extra-pair fertilizations to female red-winged blackbirds. *Proceedings of the Royal Society London, Series B*, **258**, 315–320.

Weatherhead PJ, Boag PT (1995) Pair and extra-pair mating success relative to male quality in red-winged blackbirds. *Behavioral Ecology and Sociobiology*, **37**, 81–91.

Webster MS, Chuang-Dobbs HC, Holmes RT (2001) Microsatellite identification of extrapair sires in a socially monogamous warbler. *Behavioral Ecology*, **12**, 439–446.

Westneat DF, Sherman PW, Morton ML (1990) The ecology and evolution of extra-pair copulations in birds. In: *Current Ornithology*, vol. 7 (ed. Power DM), pp. 331–369. Plenum Press, New York.

Wheelwright NT, Mauck RA (1998) Philopatry, natal dispersal, and inbreeding avoidance in an island population of Savannah sparrows. *Ecology*, **79**, 755–767.

Wittzell H, von Schantz T, Zoorob R, Aufffray C (1995) Rfp-Y-like sequences assort independently of pheasant Mhc genes. *Immunogenetics*, **42**, 68–71.

Zeh JA, Zeh DW (1996) The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society London, Series B*, **263**, 1711–1717.

Zeh JA, Zeh DW (1997) The evolution of polyandry II: postcopulatory defences against genetic incompatibility. *Proceedings of the Royal Society London, Series B*, **264**, 69–75.

Zelano B, Edwards SV (2002) An MHC component to kin recognition and mate choice in birds: predictions, progress, and prospects. *American Naturalist*, **160**, S225–S237.