LUND UNIVERSITY

MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler

Richardson, David; Komdeur, J; Burke, T; von Schantz, Torbjörn

Link to publication

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler

David S. Richardson[1,2,*]**, Jan Komdeur**[2]**, Terry Burke**[3]
and Torbjörn von Schantz[4]

[1]*Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[2]*Animal Ecology Group, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands*
[3]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*
[4]*Department of Animal Ecology, Lund University, SE-223 62 Lund, Sweden*

The existence and nature of indirect genetic benefits to mate choice remain contentious. Major histocompatibility complex (MHC) genes, which play a vital role in determining pathogen resistance in vertebrates, may be the link between mate choice and the genetic inheritance of vigour in offspring. Studies have shown that MHC-dependent mate choice can occur in mammal and fish species, but little work has focused on the role of the MHC in birds. We tested for MHC-dependent mating patterns in the Seychelles warbler (*Acrocephalus sechellensis*). There was no influence of MHC class I exon 3 variation on the choice of social mate. However, females were more likely to obtain extra-pair paternity (EPP) when their social mate had low MHC diversity, and the MHC diversity of the extra-pair male was significantly higher than that of the cuckolded male. There was no evidence that females were mating disassortatively, or that they preferred males with an intermediate number of MHC bands. Overall, the results are consistent with the 'good genes' rather than the 'genetic compatibility' hypothesis. As female choice will result in offspring of higher MHC diversity, MHC-dependent EPP may provide indirect benefits in the Seychelles warbler if survival is positively linked to MHC diversity.

Keywords: major histocompatibility complex; extra-pair paternity; mate choice; sexual selection; genetic benefits

1. INTRODUCTION

Indirect genetic benefits have long been evoked to explain active mate choice in situations where direct benefits do not appear to be gained; for example, where females in socially monogamous species pursue extra-pair copulations (Andersson 1994). However, the existence and nature of a genetic benefit remain contentious (reviewed in Jennions & Petrie 2000). Indirect genetic benefits may be gained through the acquisition of good paternal genes (Petrie 1994; Hasselquist *et al.* 1996) or the enhanced genetic compatibility of maternal and paternal genomes (Zeh & Zeh 1996; Brown 1997; Tregenza & Wedell 2000). Several studies have provided evidence that mate choice can increase offspring fitness through genetic benefits (Petrie 1994; Hasselquist *et al.* 1996; Wilkinson *et al.* 1998). However, very few have identified the underlying genes that may account for variation both in the trait upon which choice is made and in fitness variation (von Schantz *et al.* 1996, 1997; Wilkinson *et al.* 1998).

The major histocompatibility complex (MHC) is an important component of the vertebrate acquired immune system. Molecules encoded by the MHC alleles bind peptides, and when these peptides are derived from foreign antigens, the molecule and bound peptide form a complex with T cells, thereby triggering an adaptive immune response (Hughes & Yeager 1998). Individual

differences in MHC diversity influence pathogen susceptibility (Doherty & Zinkernagel 1975), and the selection pressures exerted on a population by pathogens are thought to be important in maintaining MHC polymorphism through a number of mechanisms (Potts & Slev 1995; Jeffery & Bangham 2000). The heterozygote advantage hypothesis proposes that individuals heterozygous at MHC loci are at an advantage as they are able to detect and combat a wider range of pathogens than homozygotes (Doherty & Zinkernagel 1975). The rare allele advantage hypothesis suggests that individuals with a rare MHC allele might respond better to new pathogen variants that have evolved to evade common MHC alleles and that polymorphism is maintained through frequency-dependent selection (Bodmer 1972). Finally, temporal and/or geographical variation in the type and prevalence of pathogens may occur, resulting in fluctuations in the intensity of selection exerted by pathogens that may, in turn, drive MHC diversity.

In a population under selection pressure from pathogens, MHC-based mate choice (either on condition-dependent traits or directly on the MHC) could increase the pathogen resistance and, consequently, the fitness of offspring (Grob *et al.* 1998; Jordan & Bruford 1998; Penn & Potts 1999). Under a good-genes model, if females preferentially mate with superior-condition males that are relatively disease resistant, they may provide their offspring with 'good' MHC haplotypes associated with improved resistance to pathogens within the current

* Author for correspondence (david.richardson@uea.ac.uk).

environment, or avoid 'bad' MHC haplotypes associated with deleterious effects (Abplanalp *et al.* 1992; Wedekind *et al.* 1996; Rulicke *et al.* 1998). In this scenario, males with a greater diversity of MHC alleles should, through either heterozygote advantage or from an increased chance of having specific resistant alleles, be in better condition and, therefore, be preferred by females. It is also possible that females may directly assess the MHC of potential mates and choose to mate with diverse males, which will, in turn, sire more MHC-diverse offspring.

Alternatively, females may seek the best combination of maternal and paternal genes (Zeh & Zeh 1996; Brown 1997; Tregenza & Wedell 2000). In this case, MHC-based mating preferences may be beneficial if MHC-disassortative mating results in MHC-diverse offspring, which respond to a greater variety of pathogens (Potts & Slev 1995; Hedrick 2002; Penn *et al.* 2002). MHC-dissimilar mates may also provide a 'moving target' against rapidly evolving parasites that escape immune recognition (Penn & Potts 1999). Other studies have suggested that an intermediate, rather than the highest, level of MHC diversity is optimal (Wegner *et al.* 2003, 2004; Kurtz *et al.* 2004; but see Hedrick 2004); consequently, the preferred male may be the one that results in an intermediate amount of MHC diversity in the offspring (Penn & Potts 1999; Aeschlimann *et al.* 2003).

Various studies have provided evidence that MHC-based mate choice occurs in mammals and fishes (Yamazaki *et al.* 1988; Potts *et al.* 1991; Ober *et al.* 1997; Wedekind & Furi 1997; Penn & Potts 1999; Reusch *et al.* 2001; Aeschlimann *et al.* 2003), though some other studies have found no such patterns (Hedrick & Black 1997; Paterson & Pemberton 1997; Wedekind *et al.* 2004). Although birds have been used extensively for studies of sexual selection and mate choice, little work has focused on the role of the MHC (von Schantz *et al.* 1996, 1997). Extra-pair paternity (EPP) occurs in the majority of passerine bird species (Birkhead & Møller 1992; Griffith *et al.* 2002) and may be a strategy used by females that are restricted in their choice of social mate to improve the genetic quality of their offspring (reviewed in Jennions & Petrie 2000; Tregenza & Wedell 2000). MHC-based extra-pair mating may be important in passerines, but very few studies have addressed this question so far (Freeman-Gallant *et al.* 2003; Westerdahl 2004).

The cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*) provides an excellent opportunity to test MHC-dependent mating patterns in a wild bird species. Pair-bonded adults are socially monogamous (Komdeur 1992), but social mate choice is extremely restricted, as opportunities to pair up rarely occur owing to a combination of restricted habitat, life-long social fidelity and the relatively long lifespan of this species (Komdeur 1992). EPP accounts for approximately 40% of offspring (Richardson *et al.* 2002). The Seychelles warbler also contains limited genetic variation at the MHC (thus making statistical analysis more tractable), though there is evidence, such as the high non-synonymous to synonymous substitutions ratio in the peptide-binding region (PBR) of the class I MHC, that this variation is maintained by selection (Richardson & Westerdahl 2003). The reduced number of MHC genotypes in this population (Richardson & Westerdahl 2003; Hansson &

Richardson 2004) also means that individuals often encounter MHC-similar potential mates and are, therefore, more likely to have evolved to discriminate against such matings.

In this study, we screened MHC class I exon 3 sequence variation for Seychelles warblers present on Cousin Island between 1997 and 1999. By combining the MHC genotype data with the demographic and parentage data available (Richardson *et al.* 2001, 2002), we were able to investigate both MHC-based social and extra-pair mating patterns. We predict that the highly restricted nature of social mate choice will prevent MHC-based social mate choice but will, conversely, favour the evolution of MHC-based extra-pair mate choice. This is, to our knowledge, the first study that has been able to compare directly the MHC genotypes of the within-pair and extra-pair mates in an avian species. We specifically tested whether females mated preferentially with: (i) more MHC-dissimilar mates, (ii) more MHC-diverse males or (iii) males that provided an intermediate optimum level of MHC diversity for offspring.

2. METHODS

(a) *Study site and population*

The entire population of Seychelles warblers on Cousin Island was monitored between 1997 and 1999. During this time, almost all birds were individually colour-ringed using a unique combination of three colour rings and a British Trust for Ornithology metal ring. There is extremely limited migration on or off the island (Komdeur *et al.* 2004) and all breeding attempts were monitored; therefore the complete life history, status and putative pedigree of nearly all birds were known. In each breeding season (June–September, December–February) all territories were checked for nesting activity at least once every two weeks by following the resident female for 15 min. Although warblers can breed independently in their first year, a lack of suitable independent breeding opportunities drives some individuals into becoming subordinates within their natal territory (Komdeur 1992; Richardson *et al.* 2003a,b). The status assigned to each bird was based upon field observations combined with long-term demographic data. The 'social' male and female were defined as the dominant, pair-bonded male and female while the term 'subordinate' included all other birds (more than 8 months old) that were resident in the territory.

During the study, almost all birds were ringed (approximately 96%) and blood-sampled. Blood samples (approximately 15 μl) were collected by brachial venipuncture, diluted in 800 μl of 100% ethanol in a screw-cap Microfuge tube and stored at room temperature. The Seychelles warbler usually produces one clutch per season and this normally consists of just one egg, but about 20% of nests contain two or three eggs (Komdeur 1991; Richardson *et al.* 2001). We attempted to sample all offspring produced in each breeding season. Many nests could not be reached immediately after hatching and it is possible that mortality occurred in some nests. Therefore, the offspring sampled may not have included all the offspring that hatched, but offspring that were found dead in the egg or nest are included in this analysis. Dead embryos that failed to hatch were extracted from eggs and stored in 100% ethanol. Molecular sexing using the PCR method devised by Griffiths *et al.* (1998) was used to confirm the sex of each individual.

Territory quality was determined in each breeding season following Komdeur (1994). Mass, tarsus length and wing length were measured for all birds.

Subordinate females within a territory may also sometimes breed by laying an egg in the primary female's nest (Richardson *et al.* 2001). However, subordinate females were not included in the analysis of social and extra-pair mate choice for a number of reasons. First, only primary females, which were pair-bonded with the primary male in the territory, could be counted as having made a social mate-choice decision. Subordinate females, which normally remain in their natal territory, do not show close pair bonds with the territorial male (J. Komdeur and D. Richardson, personal observation). Second, a consequence of natal philopatry is that subordinate females are often related to the male in the territory, and the high levels of EPP gained by these subordinates may possibly be a mechanism to avoid inbreeding (Richardson *et al.* 2004). Third, as only approximately 30% of territories contained subordinate females, and only approximately half of these breed, sample sizes were very low and precluded any meaningful analysis.

(b) *Parentage, relatedness and heterozygosity*

Individuals were genotyped using 14 polymorphic microsatellite markers isolated in the Seychelles warbler (Richardson *et al.* 2000). Parentage had previously been determined, following Richardson *et al.* (2001), for all offspring (Richardson *et al.* 2002). Mean individual heterozygosity was calculated across all microsatellite loci (see Richardson *et al.* 2003*a*). Individual pairwise coefficients of relatedness (*r*) based on microsatellite genotype similarity were calculated between all birds using the KINSHIP program (Goodnight & Queller 1999). The mean pairwise relatedness between all random individuals in the population was set to zero.

(c) *MHC screening*

A protocol for motif-specific amplification using PCR, followed by the separation of sequences using denaturing gradient gel electrophoresis (DGGE), was used to screen MHC class I exon 3 sequence variation (Richardson & Westerdahl 2003; Westerdahl *et al.* 2004). The MHC class I molecules are expressed on nearly all nucleated cells, where they present peptides from intracellular pathogens (e.g. viruses; Hughes & Yeager 1998). Exon 3 was chosen for this study as it encodes the PBR of the MHC molecule, the region that is critical for the binding of peptides and for generating specific immune responses when the peptide bound is non-self (Hughes & Yeager 1998). The class I exon 2, which was not screened in this study, also encodes part of the PBR, and it is possible that critical variation in the PBR may have been missed. We therefore acknowledge that this study is limited and the results (null or otherwise) will not exclude the possibility that other regions of the MHC, i.e. class I exon 2 or, indeed, class II loci could influence mate choice.

The sequence-specific amplification detects a limited number of sequences from several class I loci. A total of 10 different DGGE bands, each representing a unique sequence (and a unique amino acid sequence), were detected in the Seychelles warbler population (Richardson & Westerdahl 2003). The sequences, which cannot be assigned to specific loci, are hereafter termed MHC bands. The screening method detects two to seven MHC bands per individual and gives a broad estimate of the MHC class I exon 3

variation present within each bird. Individuals that have more MHC-bands are likely to be heterozygous at more loci than individuals with fewer MHC-bands; the number of MHC-bands within an individual (hereafter referred to as MHC diversity) will therefore be an indicator of heterozygosity. The method is also repeatable, more sensitive in detecting genetic variation than RFLP methods, and enables us to avoid screening pseudogenes (Westerdahl *et al.* 2004).

The MHC genotypes were used to confirm the parentage of the previously assigned chicks. In total, 122 chicks (86 within-pair offspring, 36 extra-pair offspring) for which the genetic parents were MHC genotyped were included in the analysis. The genetic father was not the territorial male in cases of EPP. In all these cases, except two, the MHC genotype of the territorial male was also known. For females that produced multiple offspring (either in the same year or over different breeding attempts), the mean MHC band-sharing and MHC diversity were calculated separately for all within-pair and EPP, respectively. Thirteen females gained both within-pair and EPP in different years. The final dataset represented the social ($N=53$) or extra-pair ($N=31$) mate choice of primary female birds over a period of 3 years.

MHC band-sharing coefficients were calculated using the formula $S = 2N_{ab}/(N_a + N_b)$ (Wetton *et al.* 1987) for (i) social mate dyads, (ii) genetic mate dyads and (iii) dyads of random pairs. The latter dyads involved pairing each MHC-genotyped female with a single randomly chosen MHC-genotyped adult male from the population (with each male used only once), and were used to calculate the mean and frequency distribution parameters for random pair band-sharing.

(d) *Statistical analysis*

Logistic regression analysis was used to determine whether the occurrence of EPP was influenced by the MHC. EPP was fitted as the response variate. The MHC diversity of the female and territorial male (and the interaction between them), and the MHC band-sharing between the two, were included as explanatory variables. All explanatory terms were initially entered into the model and the significance of each explanatory term was assessed by its Wald statistic. The minimum adequate model is presented following parsimonious streamlining (the stepwise backwards removal of non-significant terms) of the original model.

Male size, body condition and territory quality may be influenced by the male's MHC and could, therefore, be the direct cause of any link between EPP and male MHC characteristics. Univariate tests, using all the males from the Cousin Island population for which appropriate data were available, were therefore used to test if MHC diversity correlated with these other variables.

Transformations and non-parametric tests were used where appropriate. The probabilities (referred to as the power) of detecting a large, medium or small effect are given for tests with non-significant results. The large, medium or small effect sizes are set at 0.80, 0.50 and 0.20 for tests of means, at 0.50, 0.30 and 0.10 for correlations and at 0.35, 0.15 and 0.02 for the logistic regression analysis (following the conventions suggested by Cohen 1988). A *post hoc* power analysis was performed using GPOWER (Faul & Erdfelder 1992). All other tests were performed using SPSS 10.7 (SPSS Inc. 1999). All tests are two-tailed and corrected for
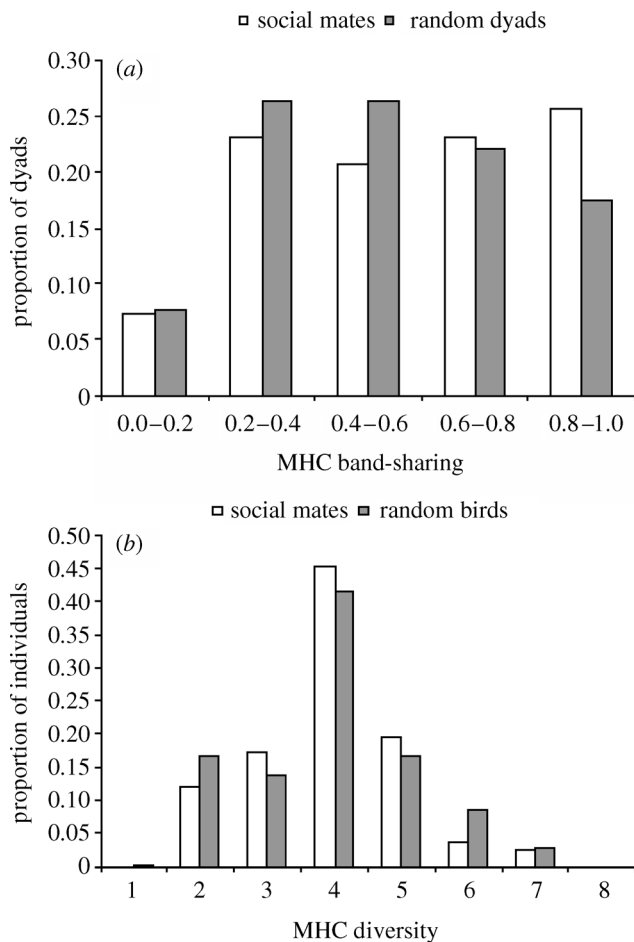
Figure 1. The MHC characteristics of the social mate of female Seychelles warblers. The proportional distribution of (*a*) MHC band-sharing between the social mate and female compared with MHC band-sharing between random male–female dyads in the population, (*b*) MHC diversity of the social mate compared with that of random birds in the population.

continuity or tied ranks, as appropriate. Means are given ± s.d.

3. RESULTS

(a) *MHC variation*
Both MHC and microsatellite genotypes were available for a total of 471 individuals. The MHC band-sharing between females and males in the population was normally distributed around a mean of 0.56 ± 0.27 (Kolmogorov–Smirnov $Z = 0.98$, d.f. $= 235$, $p = 0.29$). Individual MHC diversity was not normally distributed (mean $= 3.90 \pm 1.26$; Kolmogorov–Smirnov $Z = 0.11$, d.f. $= 471$, $p < 0.01$, probably as a consequence of the MHC screening method detecting multiple loci. Trans-formations (including a Box–Cox normality; best lambda $= 0.37$) were unable to improve the data's approximation to a normal distribution. There was no difference between the MHC diversity of males and females in the population (median $= 4$ versus 4, range $= 2$–7 versus 1–7, $N = 237$ versus 235, respectively; Mann–Whitney $U = 27\,373.0$, $p = 0.74$; power $= 1.00$, 1.00, 0.70). When calculated for all breeding pairs, MHC band-sharing was significantly positively related to pairwise relatedness ($F_{1,96} = 6.06$, $p = 0.02$).
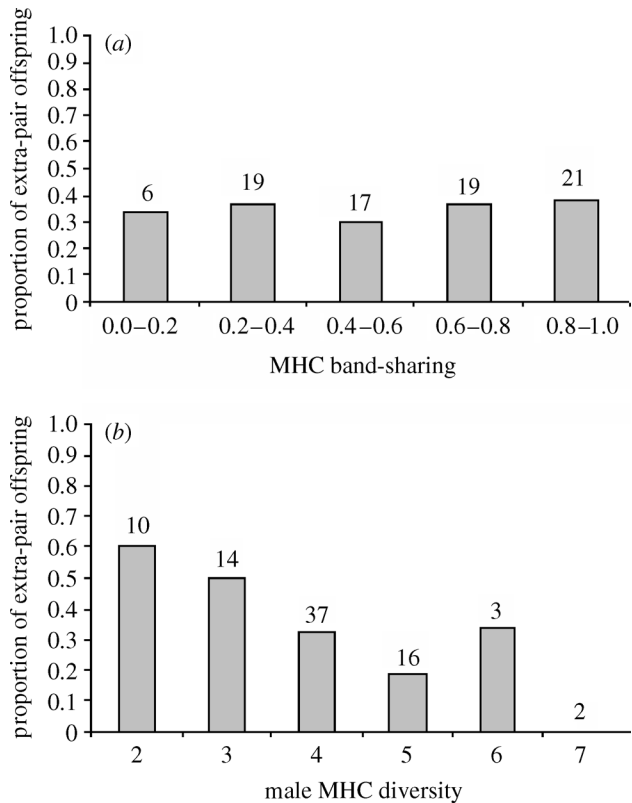
Figure 2. Extra-pair paternity and the MHC characteristic of the social male. The proportional frequency of extra-pair paternity in relation to: (*a*) the MHC band-sharing between the social male and female, (*b*) the MHC diversity of the social male. Figures above the columns give the number of independent females for which MHC choice was identified in each class.

(b) *Social mate choice*
Figure 1 shows the proportional distribution of: (*a*) MHC band-sharing for social mates and for random dyads of birds within the population and (*b*) MHC diversity of social pair males versus all birds in the population. There was no significant difference in the mean or variance in band-sharing of social mates compared with random dyads ($N = 82$ versus 235, mean $= 0.56 \pm 0.27$ versus 0.58 ± 0.29, respectively; $t = 0.82$, d.f. $= 315$, $p = 0.41$; power $= 1.00$, 0.97, 0.34; Levene's test for equality of variances, $F = 1.27$, $p = 0.26$; figure 1*a*). The median MHC diversity of social pair males did not differ from the population median (median $= 4$ versus 4, range $= 2$–7 versus 1–7, $N = 82$ versus 472, respectively; Mann–Whitney $U = 19061$, $p > 0.82$; power $= 1.00$, 0.99, 0.51; figure 1*b*). There was also no correlation between the MHC diversity of the female and her social mate (Spearman's rho, $r = 0.13$, $N = 82$, $p = 0.23$; power $= 1.00$, 1.00, 0.88, 0.26).

(c) *Extra-pair paternity*
Figure 2 shows the proportion of offspring that were extra-pair in relation to the MHC characteristics of the social male. EPP appeared to remain constant across the different levels of MHC band-sharing between the female and her social male (figure 2*a*), but decreased as a function of male MHC diversity (figure 2*b*). In neither case was

Table 1. Logistic regression analysis showing the effect of MHC variation on extra-pair paternity.
(The Wald test statistic and level of significance are shown for all terms included in the original model. Terms remaining in the minimum model are shown in bold; predicted effect sizes and standard errors are given in the minimal model (d.f. = 1, 80; $R^2 = 0.10$).)

explanatory variables	Wald	d.f.	p
female MHC diversity	0.35	1	0.56
social male MHC diversity	**5.43**	**1**	**0.02**
female–social male MHC band-sharing	0.39	1	0.54
female MHC diversity × social male MHC diversity	0.21	1	0.15
minimal model	average effect		s.e.
constant	1.54		0.93
social male MHC diversity	−0.56		0.24

there any suggestion of an intermediate optimum for the MHC characteristic in relation to EPP.

Logistic regression analysis was then used to determine statistically whether EPP was influenced by the MHC (table 1). Social male MHC diversity was significantly negatively related to the occurrence of EPP; females were more likely to gain EPP when paired to a social mate of low MHC diversity. Neither the female's MHC diversity nor the MHC band-sharing between the female and her social mate significantly influenced the occurrence of EPP. The interaction between the female's and the social male's MHC diversity, added to the model to test whether the combination of the female's and social male's MHC diversity affected the occurrence of EPP, had no significant effect on the model. Pairwise relatedness between the social male and female was not included in the original model as it was positively correlated with MHC band-sharing. When added to the final model, pairwise relatedness had no significant effect on the variance explained (Wald = 0.07, d.f. = 1, $p = 0.80$). The power of the logistic regression to detect a large, medium or small effect was calculated as 1.00, 0.88 and 0.19, respectively.

The MHC diversity values of the female and her genetic mate were not correlated (Spearman's rho, $r = 0.13$, $N = 84$, $p = 0.22$, power = 1.00, 0.89, 0.23). Pairwise tests were then performed, using the 29 EPP cases for which MHC measures for both the social and extra-pair male were available. The mean female–extra-pair male band-sharing was not significantly different from mean female–social male band-sharing (0.56 ± 0.29 versus 0.60 ± 0.29, paired t-test, $t = -0.52$, d.f. = 28, $p = 0.61$; power = 0.84, 0.45, 0.11; figure 3a). Also, the variance in female–male band-sharing was not less with extra-pair than with social mates (Levene's test for equality of variances, $F = 0.034$, $p = 0.86$). The MHC diversity of extra-pair males was significantly higher than that of the social males they cuckolded (4.11 ± 1.47 versus 3.52 ± 1.06, respectively, $N = 29$; Wilcoxon signed-rank test, $Z = -1.93$, $p = 0.05$; figure 3b). Interestingly, the extra-pair mates of females that gained EPP had the same level of MHC diversity as the within-pair mates of those females that remained faithful (within-pair paternity (WPP) versus EPP; 4.13 ± 1.07
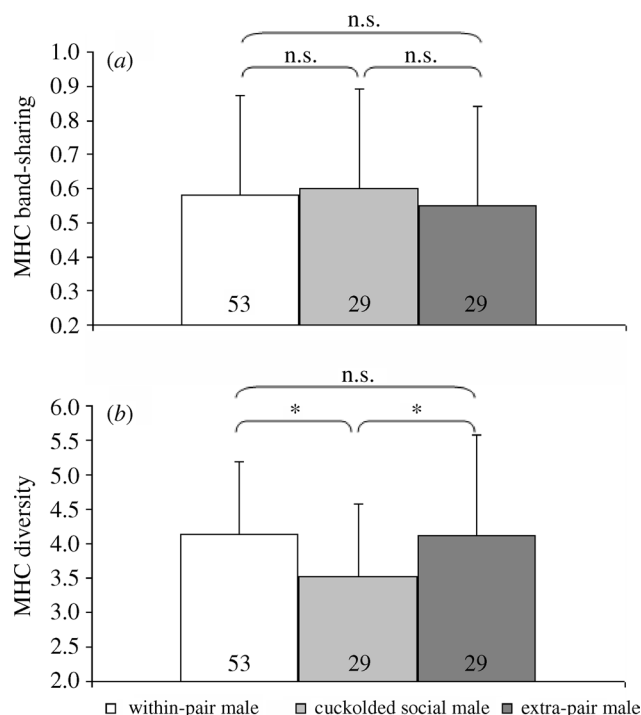
Figure 3. The MHC characteristics of the social and genetic mate of female Seychelles warblers in relation to extra-pair paternity. (a) MHC band-sharing between the male and female, (b) MHC diversity of the male. Non-cuckolded social males, open columns; cuckolded social male, lightly shaded columns; extra-pair male, heavily shaded columns. Error bars represent 1 s.d. *$p < 0.05$. Sample sizes are shown as numbers in the columns. As the MHC of the cuckolded social male was not known for two of the EPP cases, the pairwise comparison between the cuckolded social males and the extra-pair male was based on 29 cases (see text for statistical details).

($N = 53$) versus 4.11 ± 1.47 ($N = 31$); t-test, $t = 0.05$, d.f. = 82, $p = 0.96$; power = 0.94, 0.59, 0.14). The final MHC band-sharing of within-pair and extra-pair genetic mates also did not differ; 0.58 ± 0.29 ($N = 53$) versus 0.55 ± 0.29 ($N = 31$); t-test, $t = 0.44$, d.f. = 82; $p = 0.66$; power = 0.94, 0.59, 0.14).

(d) Offspring

The MHC diversity of nestlings produced through within-pair and EPP did not differ significantly (WPP versus EPP, 3.93 ± 1.13 ($N = 53$) versus 3.97 ± 1.14 ($N = 31$); $U = 790.50$, $p = 0.76$; power = 0.97, 0.71, 0.22). However, to determine whether females use EPP to gain more MHC diverse offspring, the MHC diversity that offspring would have had if the female had mated with the social male in the territory needs to be compared with the MHC diversity of the extra-pair offspring. The predicted offspring MHC diversity could not be calculated from the population-wide regression of offspring against parent MHC diversity, as the errors of this regression were not normally distributed. However, the MHC diversity of offspring was strongly correlated with that of both the mother (Spearman's rho, $r = 0.43$, $N = 84$, $p < 0.001$) and father (Spearman's rho, $r = 0.34$, $N = 84$, $p < 0.001$). Consequently, the offspring that cuckolded social males with low MHC diversity would have produced, had they not been cuckolded, would have had lower MHC diversity than the offspring produced

by the higher MHC diversity extra-pair males. There was no correlation between the MHC diversity of the female and her genetic mate (Spearman's rho, $r=0.13$, $N=84$, $p=0.22$; power=1.00, 0.89, 0.23) and offspring MHC diversity was not correlated with mother–father band-sharing (Spearman's rho, $r=-0.14$, $N=100$, $p<0.16$; power=1.00, 0.93, 0.26).

(e) *Correlates of MHC diversity*
Traits that may be associated with male quality were then tested for a relationship with MHC diversity to determine whether they could be the direct cause of the link between extra-pair mate choice and MHC diversity. There was no correlation between MHC diversity and tarsus (Spearman's rho=-0.02, $N=158$, $p=0.78$; power=1.00, 0.99, 0.35) or wing length (Spearman's rho=-0.06, $N=156$, $p=0.46$; power=1.00, 0.99, 0.35), but there was a weak negative correlation with weight (Spearman's rho=-0.162, $N=154$, $p=0.05$). A body condition index derived from the regression of weight on tarsus also showed a negative correlation with MHC diversity (Spearman's rho, $r=-0.18$, $N=153$, $p=0.027$). Territory quality was not related to the MHC diversity of the territory male (Spearman's rho=-0.00, $N=102$, $p=0.93$; power=1.00, 0.93, 0.26).

4. DISCUSSION
(a) *MHC-based mate choice*
There was no evidence that MHC-based social mate choice occurred in primary female Seychelles warblers. MHC-disassortative pairing, with females preferring males with which they had lower levels of band-sharing, did not appear to occur (there was no difference in the mean band-sharing between females and their social mates compared with random males in the population). There was also no evidence that females prefer social mates with whom they had intermediate levels of MHC band-sharing as the variance in female–male band-sharing was not significantly less when comparing social males with random males (figure 1a). Finally, there was no negative correlation between the MHC diversity of the social-pair male and female, as expected if the female was seeking a social mate, who, in combination with her, would provide offspring with an intermediate optimal number of MHC alleles.

There was, however, evidence of MHC-based extra-pair mating. Extra-pair matings did not appear to be either MHC disassortative, nor biased towards genetic mates with an intermediate level of band-sharing (both the mean and variance in band-sharing were not significantly different between extra-pair males and social males), but were positively associated with male MHC diversity. EPP occurred where the MHC diversity of the social mate was lower than in cases of within-pair paternity. Furthermore, the MHC diversity of the extra-pair male was significantly higher than that of the cuckolded social-pair male. There was no negative correlation between the MHC diversity of the extra-pair male and female, as expected if females were choosing males to produce offspring with an intermediate number of alleles.

Although MHC-based mate choice has been shown in other vertebrates (see above), little work has been done with regard to the role of the MHC in birds. An exception

to this was a study of pheasants, *Phasianus colchicus*, in which it was found that females preferred males with larger spurs, and that spur length was associated with MHC genotype (von Schantz *et al.* 1997). In passerines, very recent work suggests a role for the MHC in both within- and extra-pair mate choice in the savannah sparrow, *Passerculus sandwichensis* (Freeman-Gallant *et al.* 2003). In this species, young females (but not older birds) appeared to prefer MHC-dissimilar social mates, and high MHC similarity between social mates (all ages) was linked to the occurrence of EPP. However, this study used a crude measure of MHC variability that may have been confounded by being linked to genome-wide variability. Furthermore, extra-pair males were not identified, so it was impossible to ascertain whether or not the females actually gained EPP from more MHC-dissimilar males. Another study which used the same, more sophisticated MHC screening method as in the present study, found no evidence that MHC influenced social mate choice in the great reed warbler, *Acrocephalus arundinaceus*, despite having considerable statistical power (Westerdahl 2004).

In the Seychelles warbler, social mate choice did not appear to be influenced by the MHC. This is, perhaps, not surprising considering the extreme constraints imposed upon social mate choice in this species. The Cousin Island population contains a surplus of adult birds (320 birds for circa 100 territories Komdeur 1992, 2003; Richardson *et al.* 2002) and this, combined with the fact that individuals pair for life, defend year-round territories and have a relatively long lifespan (Komdeur 1992), means that independent breeding opportunities occur rarely. In fact, a substantial proportion of adult birds die without ever having acquired a breeding territory (Komdeur *et al.* 2004). Under these conditions, unpaired females should pair with any male possessing a breeding territory, irrespective of his individual quality. Females that are constrained in their social mate choice could, however, improve the genetic quality of their offspring by gaining EPP (reviewed in Jennions & Petrie 2000; Tregenza & Wedell 2000). In the Seychelles warbler, EPP does appear to be influenced by male MHC diversity. However, the mechanisms and benefits that drive the pattern are as yet unknown.

(b) *Mechanisms driving MHC-based mate choice*
Non-random patterns of MHC mating may be caused by mechanisms other than direct MHC-based mating. For example, inbreeding avoidance may prevent mating between closely related individuals and lead to a pattern of MHC-disassortative mating. In the Seychelles warbler, we found no patterns of mating (disassortative or otherwise) in relation to MHC band-sharing, a measure that should reflect kinship between individuals. Also, pairwise relatedness did not affect the occurrence of EPP in this study. Finally, previous work on the Seychelles warbler (using the same dataset) has shown that neither social mate choice or extra-pair mate choice are affected by patterns of pairwise relatedness (Richardson *et al.* 2004), and the high level of EPP observed (Richardson *et al.* 2001) is not part of a strategy used by primary females to avoid inbreeding. The patterns of MHC-based extra-pair mate choice seen in the present study do not, therefore, appear to be due to inbreeding avoidance.

Mate choice can be based directly on an individual's MHC. For example, olfactory cues may be used to assess the MHC composition of potential partners and, subsequently, to choose a mate (Potts *et al.* 1991; Wedekind & Furi 1997; Landry *et al.* 2001; Reusch *et al.* 2001; Aeschlimann *et al.* 2003). Despite popular belief to the contrary, olfactory cues can be important in birds (Ioale & Papi 1989; Clark *et al.* 1993; Hagelin *et al.* 2003) and smell could be used to assess the MHC of potential partners. However, given that mating in the Seychelles warbler is biased towards males that are MHC-diverse (rather than MHC-dissimilar or an MHC-intermediate), the most parsimonious hypothesis may be that there is an indirect link between the MHC and mate choice. For example, if mate choice is based upon condition-dependent cues and MHC diversity influences condition (e.g. as for spur length in pheasants; von Schantz *et al.* 1996, 1997), then female Seychelles warblers may be mating with higher MHC diversity males as a result of choosing extra-pair males with superior traits. Further work is required to investigate the cues that underlie the patterns observed in the Seychelles warbler, but initial analysis shows that three obvious traits that could reflect male genetic quality (tarsus length, wing length and territory quality) were not correlated with male MHC diversity. Surprisingly, mass (and body condition) were negatively correlated with MHC diversity. This relationship, which is in the opposite direction to that predicted based on the idea that MHC-diverse males are of better quality, needs to be investigated further.

The MHC-based extra-pair matings observed in the Seychelles warbler may, on the other hand, be a result of sperm competition (reviewed in Birkhead & Møller 1998; Wedekind *et al.* 2004). Females may gain extra-pair copulations from males regardless of their MHC genotype. Sperm competition may then favour sperm from males of high MHC diversity. This may act through male–male sperm competition, if sperm quality or quantity is influenced by male MHC diversity. Alternatively, the MHC-based extra-pair mating pattern may be a result of cryptic female choice—there is evidence that MHC antigens may be expressed on sperm and that non-random fusion of gametes can occur (reviewed in Dorak *et al.* 2002; Wedekind *et al.* 2004). Both mechanisms could lead to a situation where, on average, EPP occurs more often in females that are mated socially to males with low genetic diversity, and the resulting genetic fathers will then have higher MHC diversity than the cuckolded social mates.

It is important to note that the above MHC-based mating pattern is caused by the social mate being of lower than average MHC diversity, while the extra-pair male has MHC diversity equal to the population mean (and to the MHC diversity of the non-cuckolded primary males). To us, this evidence does not suggest that the female is seeking out the 'best' (above average) high MHC diversity males in the population for extra-pair matings. Rather, we suggest a scenario in which females may copulate with various extra-pair males and that males with lower than average MHC diversity are either less able to guard their paternity, or are at a disadvantage in sperm competition. There is some evidence that females discriminate between possible extra-pair mates, i.e. from field observations (unpublished data) and the fact that extra-pair males are nearly always primary males from other territories (Richardson *et al.* 2002). However, this last result may be explained by subordinate males being hormonally suppressed (van de Crommenaker *et al.* 2004). Direct mate choice and post-copulatory sperm competition experiments are required to resolve these issues.

(c) *MHC and fitness*

Increased MHC diversity has been both theoretically and empirically linked to increased pathogen resistance and survival (Potts & Slev 1995; Hedrick 2002; Penn *et al.* 2002). Indeed, strong pathogen-mediated balancing selection is thought to be responsible for maintaining the remarkably high levels of variation observed at several MHC loci in the otherwise genetically monomorphic endemic San Nicolas Island fox, *Urocyon littoralis dickeyi* (Aguilar *et al.* 2004). In small, bottlenecked populations where MHC diversity may be reduced—as is the case in the Seychelles warbler (Richardson & Westerdahl 2003)—behavioural strategies maximizing the MHC diversity may be especially important (Aeschlimann *et al.* 2003). Other studies have shown that individuals may maximize the MHC diversity of their offspring by mating disassortatively (Potts *et al.* 1991; Wedekind & Furi 1997), or that they may optimize it by 'allele counting' (Reusch *et al.* 2001; Aeschlimann *et al.* 2003). Our study found no evidence that Seychelles warblers use either of these strategies (though it should be noted that the power of the analysis to detect small effects was limited and so null results must be treated with some caution). Instead, Seychelles warbler females appear to gain EPP with males of higher MHC diversity when socially mated to low MHC diversity males. As there is a positive correlation between paternal and offspring MHC diversity, this pattern of EPP will result in offspring of higher MHC diversity. MHC-based EPP may, therefore, provide indirect benefits in the Seychelles warbler if (as suggested above) survival is positively linked to MHC diversity.

REFERENCES

Abplanalp, H., Sato, K., Napolitano, D. & Reid, J. 1992 Reproductive performance of inbred congenic leghorns carrying different haplotypes for the major histocompatibility complex. *Poult. Sci.* **71**, 9–17.

Aeschlimann, P. B., Haberli, M. A., Reusch, T. B. H., Boehm, T. & Milinski, M. 2003 Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. *Behav. Ecol. Sociobiol.* **54**, 119–126.

Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D. & Wayne, R. K. 2004 High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proc. Natl Acad. Sci. USA* **101**, 3490–3494.

Andersson, M. B. 1994 *Sexual selection*. Princeton: Princeton University Press.

Birkhead, T. R. & Møller, A. P. 1992 *Sperm competition in birds: evolutionary causes and consequences*. London: Academic Press.

Birkhead, T. R. & Møller, A. P., 1998 *Sperm competition and sexual selection*. London: Academic Press.

Bodmer, W. 1972 Evolutionary significance of the HLA sytem. *Nature* **237**, 139–145.

Brown, J. L. 1997 A theory of mate choice based on heterozygosity. *Behav. Ecol.* **8**, 60–65.

Clark, L., Avilova, K. V. & Bean, N. J. 1993 Odor thresholds in passerines. *Comput. Biochem. Physiol.* **104**, 305–312.

Cohen, J. 1988 *Statistical power analysis for the behavioral sciences*. New Jersey: Erlbaum.

Doherty, P. & Zinkernagel, R. 1975 Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature* **256**, 50–52.

Dorak, M. T., Lawson, T., Machulla, H. K. G., Mills, K. I. & Burnett, A. K. 2002 Increased heterozygosity for MHC class II lineages in newborn males. *Genes Immun.* **3**, 263–269.

Faul, F. & Erdfelder, E. 1992 GPOWER: a priori, post-hoc, and compromise power analyses for ms-dos. Bonn, Germany: Bonn University, Department of Psychology.

Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. 2003 Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Mol. Ecol.* **12**, 3077–3083.

Goodnight, K. F. & Queller, D. C. 1999 Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol.* **8**, 1231–1234.

Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998 A DNA test to sex most birds. *Mol. Ecol.* **7**, 1071–1075.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002 Extra-pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* **11**, 2195–2212.

Grob, B., Knapp, L. A., Martin, R. D. & Anzenberger, G. 1998 The major histocompatibility complex and mate choice: inbreeding avoidance and selection of good genes. *Exp. Clin. Immunogenet.* **15**, 119–129.

Hagelin, J. C., Jones, I. L. & Rasmussen, L. E. L. 2003 A tangerine-scented social odour in a monogamous seabird. *Proc. R. Soc. B* **270**, 1323–1329.

Hansson, B. & Richardson, D. S. 2005 Genetic variation in two endangered *Acrocephalus* species compared to a widespread congener: estimates based on functional and random loci. *Anim. Conserv.* **8**, 83–90.

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.

Hedrick, P. W. 2002 Pathogen resistance and genetic variation at MHC loci. *Evolution* **56**, 1902–1908.

Hedrick, P. W. 2004 Comment on 'Parasite selection for immunogenetic optimality'. *Science* **303**, 957.

Hedrick, P. W. & Black, F. L. 1997 HLA and mate selection: no evidence in South Amerindians. *Am. J. Hum. Genet.* **61**, 505–511.

Hughes, A. L. & Yeager, M. 1998 Natural selection at major histocompatibility complex loci of vertebrates. *Annu. Rev. Genet.* **32**, 415–435.

Ioale, P. & Papi, F. 1989 Olfactory bulb size, odor discrimination and magnetic insensitivity in hummingbirds. *Physiol. Behav.* **45**, 995–999.

Jeffery, K. J. M. & Bangham, C. R. M. 2000 Do infectious diseases drive MHC diversity? *Microbes Infect.* **2**, 1335–1341.

Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**, 21–64.

Jordan, W. & Bruford, M. 1998 New perspectives on mate choice and the MHC. *Heredity* **81**, 127–133.

Komdeur, J. 1991 Cooperative breeding in the Seychelles warbler. *Department of Zoology.*

Komdeur, J. 1992 Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature* **358**, 493–495.

Komdeur, J. 1994 Experimental evidence for helping and hindering by previous offspring in the cooperatively-breeding Seychelles warbler *Acrocephalus sechellensis*. *Behav. Ecol. Sociobiol.* **34**, 175–186.

Komdeur, J. 2003 Daughters on request: about helpers and egg sexes in the Seychelles warbler. *Proc. R. Soc. B* **270**, 3–11.

Komdeur, J., Piersma, T., Kraaijeveld, K., Kraaijeveld-Smit, F. & Richardson, D. S. 2004 Why Seychelles warblers fail to recolonize nearby islands: were they selected for reduced flight performance? *Ibis* **146**, 298–302.

Kurtz, J., Kalbe, M., Aeschlimann, P. B., Haberli, M. A., Wegner, K. M., Reusch, T. B. H. & Milinski, M. 2004 Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. *Proc. R. Soc. B* **271**, 197–204.

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*). *Proc. R. Soc. B* **268**, 1279–1285.

Ober, C., Weitkamp, L. R., Cox, N., Dytch, H., Kostyu, D. & Elias, S. 1997 HLA and mate choice in humans. *Am. J. Hum. Genet.* **61**, 497–504.

Paterson, S. & Pemberton, J. M. 1997 No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proc. R. Soc. B* **264**, 1813–1819.

Penn, D. J. & Potts, W. K. 1999 The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat.* **153**, 145–164.

Penn, D., Damjanovich, K. & Potts, W. 2002 MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proc. Natl Acad. Sci. USA* **99**, 11 260–11 264.

Petrie, M. 1994 Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature* **371**, 598–599.

Potts, W. K. & Slev, P. R. 1995 Pathogen-based models favoring MHC genetic diversity. *Immunol. Rev.* **143**, 181–197.

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991 Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature* **352**, 619–621.

Reusch, T. B. H., Haberli, M. A., Aeschlimann, P. B. & Milinski, M. 2001 Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **414**, 300–302.

Richardson, D. S. & Westerdahl, H. 2003 MHC diversity in two *Acrocephalus* species: the outbred great reed warbler and the inbred Seychelles warbler. *Mol. Ecol.* **12**, 3523–3529.

Richardson, D. S., Jury, F. L., Dawson, D. A., Salgueiro, P., Komdeur, J. & Burke, T. 2000 Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in *Sylviidae* species and their cross-species amplification in other passerine birds. *Mol. Ecol.* **9**, 2226–2231.

Richardson, D. S., Jury, F. L., Blaakmeer, K., Komdeur, J. & Burke, T. 2001 Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). *Mol. Ecol.* **10**, 2263–2273.

Richardson, D. S., Burke, T. & Komdeur, J. 2002 Direct benefits explain the evolution of female biased cooperative breeding in the Seychelles warblers. *Evolution* **56**, 2313–2321.

Richardson, D. S., Burke, T. & Komdeur, J. 2003a Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. *J. Evol. Biol.* **16**, 854–861.

Richardson, D. S., Komdeur, J. & Burke, T. 2003b Altruism and infidelity among warblers. *Nature* **422**, 580.

Richardson, D. S., Komdeur, J. & Burke, T. 2004 Inbreeding in the Seychelles warbler: environment-dependent maternal effects. *Evolution* **58**, 2037–2048.

Rulicke, T., Chapuisat, M., Homberger, F. R., Macas, E. & Wedekind, C. 1998 MHC-genotype of progeny influenced by parental infection. *Proc. R. Soc. B* **265**, 711–716.

Tregenza, T. & Wedell, N. 2000 Genetic compatibility, mate choice and patterns of parentage. *Mol. Ecol.* **9**, 1013–1027.

van de Crommenaker, J., Richardson, D. S., Eising, C. M., Dekker, A. L., Groothuis, T. G. G. & Komdeur, J. 2004 Testosterone, cuckoldry risk and extra-pair copulations in the Seychelles warbler. *Proc. R. Soc. B* **271**, 1023–1031.

von Schantz, T., Wittzell, H., Goransson, G., Grahn, M. & Persson, K. 1996 MHC genotype and male ornamentation: genetic evidence for the Hamilton–Zuk model. *Proc. R. Soc. B* **263**, 265–271.

von Schantz, T., Wittzell, H., Goransson, G. & Grahn, M. 1997 Mate choice, male condition-dependent ornamentation and MHC in the pheasant. *Hereditas* **127**, 133–140.

Wedekind, C. & Furi, S. 1997 Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity?. *Proc. R. Soc. B* **264**, 1471–1479.

Wedekind, C., Chapuisat, M., Macas, E. & Rulicke, T. 1996 Non-random fertilization in mice correlates with the MHC and something else. *Heredity* **77**, 400–409.

Wedekind, C., Walker, M., Portmann, J., Cenni, B., Muller, R. & Binz, T. 2004 MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *J. Evol. Biol.* **17**, 11–18.

Wegner, K. M., Kalbe, M., Kurtz, J., Reusch, T. B. H. & Milinski, M. 2003 Parasite selection for immunogenetic optimality. *Science* **301**, 1343–1343.

Wegner, K. M., Kalbe, M., Kurtz, J., Reusch, T. B. H. & Milinski, M. 2004 Response to comment on 'Parasite selection for immunogenetic optimality'. *Science* **303**, 957.

Westerdahl, H. 2004 No evidence of an MHC-based female mating preference based on genetic compatibility in the great reed warbler. *Mol. Ecol.* **13**, 2465–2470.

Westerdahl, H., Wittzell, H., von Schantz, T. & Bensch, S. 2004 MHC class I typing in a songbird with numerous loci and high polymorphism using motif-specific PCR and DGGE. *Heredity* **92**, 534–542.

Wetton, J. H., Carter, R. E., Parkin, D. t. & Walters, D. 1987 Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* **327**, 147–149.

Wilkinson, G. S., Presgraves, D. C. & Crymes, L. 1998 Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. *Nature* **391**, 276–278.

Yamazaki, K., Beauchamp, G. K., Kupniewski, D., Bard, J., Thomas, L. & Boyse, E. A. 1988 Familial imprinting determines H-2 selective mating preferences. *Science* **240**, 1331–1332.

Zeh, J. A. & Zeh, D. W. 1996 The evolution of polyandry I: intragenomic conflict and genetic incompatability. *Proc. R. Soc. B* **263**, 1711–1717.