



LUND UNIVERSITY

No evidence of an MHC-based female mating preference in great reed warblers

Westerdahl, Helena

Published in:
Molecular Ecology

DOI:
[10.1111/j.1365-294X.2004.02238.x](https://doi.org/10.1111/j.1365-294X.2004.02238.x)

2004

[Link to publication](#)

Citation for published version (APA):

Westerdahl, H. (2004). No evidence of an MHC-based female mating preference in great reed warblers. *Molecular Ecology*, 13(8), 2465-2470. <https://doi.org/10.1111/j.1365-294X.2004.02238.x>

Total number of authors:
1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

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

No evidence of an MHC-based female mating preference in great reed warblers

HELENA WESTERDAHL

Department of Animal Ecology, Ecology Building, Lund University, S-223 62 Lund, Sweden

Abstract

Female mate-choice based on genetic compatibility is an area of growing interest. The major histocompatibility complex (*MHC*) genes are likely candidates for such mate-choice since these highly polymorphic genes may both increase offspring viability and also provide direct cues for mate-choice. In great reed warblers, females actively choose a breeding partner out of a handful of males that they visit and evaluate; thus, female preference for compatible or heterozygous *MHC* genes could have evolved. Here, I investigate whether great reed warbler females preferentially mate with males with dissimilar *MHC* class I alleles or with males that are heterozygous at *MHC* class I. Despite favourable conditions, a thorough screening method and a large sample size, there was no evidence of an *MHC*-based female mating preference based on either genetic compatibility or heterozygosity in this population. Power analyses of the data sets revealed that relatively small differences (15% and 8%, respectively) between true and random pairs should have been detected.

Keywords: genetic compatibility, heterozygosity, mate-choice, *MHC*, passerine

Received 22 December 2003; revision received 21 April 2004; accepted 21 April 2004

Introduction

Females are expected to exert an active mate-choice if the benefits of such choosiness, e.g. increased viability of offspring, outweigh the costs associated with such mate-choice behaviour. The female may gain direct benefits from her choice, i.e. territory quality or nuptial food gifts, or she may receive indirect benefits through genes that confer increased offspring fitness (Andersson 1994). Indirect benefits could be gained either from mating with a male that has compatible or complementary genes (Tregenza & Wedell 2000), or from mating with a male that is in good condition because he has advantageous alleles in that environment (Hamilton & Zuk 1982). In the first case, the offspring may receive a genotype that improves survival, while in the second case the offspring may receive advantageous alleles.

The major histocompatibility complex (*MHC*), a gene complex with extreme allelic diversity, is of major importance in the immune defence (e.g. Klein 1986; Penn & Potts 1999). The *MHC* molecules bind peptides and present them to T cells and, if the bound peptide is part of a pathogen, an immune reaction will be triggered (Klein 1986; Abbas *et al.* 1994). The *MHC* polymorphism is maintained

by balancing selection, either heterozygote advantage or frequency dependent selection, and is driven by host–parasite/pathogen interactions (Bodmer 1972; Doherty & Zinkernagel 1975; Potts & Wakeland 1990; Hughes & Hughes 1995). Which *MHC* alleles are advantageous may change both over time and in space (Hedrick 2002). The evidence of balancing selection is often indirect, relying on either the ratio between nonsynonymous and synonymous substitutions or the number of *MHC* alleles per locus. So far, a limited number of studies have found direct evidence of specific *MHC* alleles/*MHC* heterozygosity improving disease resistance (e.g. Paterson *et al.* 1998; Carrington *et al.* 1999; Lohm *et al.* 2002).

The possibility that female mate-choice may be driven by genetic compatibility has recently become an area of growing interest; theory suggests that females choose mates that are genetically compatible with themselves (reviewed in Tregenza & Wedell 2000). The *MHC* is a particularly likely candidate for such mate-choice, since these highly polymorphic genes might increase offspring viability and also could be the actual cue used during mate-choice (reviewed in Penn & Potts 1999). Several mate-choice experiments in mice, and some in humans, have found that females preferentially mate with males with dissimilar *MHC* genes, though other studies failed to find a mating preference (reviewed in Penn & Potts 1999;

Correspondence: Helena Westerdahl. Fax: + 46 46 222 47 16, E-mail: Helena.Westerdahl@zooekol.lu.se

Eklund *et al.* 2000). The cue used in MHC-based mate-choice in mice and humans is thought to be based on odour preferences (Egid & Brown 1989; Ninomiya & Brown 1995; Wedekind *et al.* 1995).

In birds, female mate-choice has been investigated in many species and the list of cues that appear to be used is long; including, e.g. song repertoire, age, tail length and dancing performance (reviewed in Andersson 1994 Table 6A). So far, no studies have directly shown an MHC-based female mate-choice in birds (Zelano & Edwards 2002), although Freeman-Gallant *et al.* (2003) recently put forward evidence indicative of such a scenario in Savannah sparrows *Passerculus sandwichensis*. Furthermore, von Schantz *et al.* (1996) found that in pheasants, *Phasianus colchicus*, the male spur length was correlated with both MHC genotype and male fitness. The female pheasants preferred long-spurred males and could therefore be choosing males with a certain MHC genotype thereby gaining advantageous MHC alleles for their offspring.

The great reed warbler *Acrocephalus arundinaceus* is a polygynous long-distance migratory passerine bird. When the females arrive at the breeding grounds in the spring, males are already singing intensively from their territories to attract females. Females visit a number of males before deciding with whom to mate (Bensch & Hasselquist 1992) after taking into account the quality of the territory, the male song repertoire size and the status that she will receive in any polygynous male's harem (Bensch & Hasselquist 1991, 1992; Bensch 1996; Hasselquist 1998). Here, I examine whether great reed warbler females take MHC genes into account when choosing a mate. The two main questions are: (i) do females preferentially mate with males with dissimilar MHC class I alleles; and (ii) do females preferentially mate with males that are heterozygous at MHC class I.

Materials and methods

The study population

We studied great reed warblers at lake Kvismaren in south central Sweden (59°10' N, 15°25' E) between 1987 and 1996 (Bensch & Hasselquist 1991; Hasselquist *et al.* 1995; Hansson *et al.* 2000). The majority of the breeding males and females (> 98%) were caught in mist nets and ringed with one individual specific aluminium ring and a unique combination of two or three colour rings. During daily visits throughout the breeding season (May to July) we recorded the position of singing males, the pair formation of individual males and female nest building activity.

Female harem status

Great reed warblers have a socially polygynous mating system in which a single male has one to five females in his

harem. Female harem status is defined as follows; primary females include females of monogamous males and females of primary status in polygynous harems, secondary females include females of secondary and lower status (Westerdahl *et al.* 2000b). The first female to form a pair bond with a male, either a monogamous or a polygynous male, will become the primary female and receive more paternal care for her offspring than females of lower status (Bensch & Hasselquist 1994; Sejberg *et al.* 2000). Therefore, primary females may choose a male to receive the more advantageous primary status while females with lower status may be more prone to choosing a mate based on other conditions, including genetic quality. On average, the breeding population consisted of (\pm SD) 32 ± 6.1 breeding females and 26 ± 5.6 territorial males per year (Westerdahl *et al.* 2000b).

Female mate-choice

Females visit a number of different males (on average 5.9) before choosing a mate (Bensch & Hasselquist 1992). Males sing two different song types related to the status of the females in his territory; they sing a long song when attracting mates, and a short song when they are defending fertile females. Newly arrived females only choose mates among the males that are singing long song (Bensch & Hasselquist 1992). MHC-similarity (measured as band-sharing, see below) was calculated between each female and her mate, and between each female and all the other males with which she had the opportunity to mate (i.e. those males singing long song the day she paired with her mate).

The mate-choice analysis, based on MHC-similarity or number of MHC bands (see below), included 279 female choice events between 1987 and 1996. Each female was only recorded once per year, the first time she made a breeding attempt. Since the great reed warbler female rarely mates with the same male in successive years, each mating was regarded as an independent data point. Sixteen pairs had to be excluded (three pairs in 1987, two in 1988, seven in 1989, four in 1990) because of a lack of DNA. Two data sets were constructed to test the observed mating pattern against a random choice pattern. First, a data set of random pair combinations was generated for each female (279 female choices) by randomly selecting one male from those with whom she had the opportunity to mate. Second, a data set of the average available male was generated for the females calculated from the MHC band-sharing values of all the males with which each female (279 female choices) had the opportunity to mate.

Molecular methods

Blood samples, collected from the breeding individuals, were suspended in SET-buffer and stored at -20°C . DNA

was isolated from blood using standard phenol/chloroform-isoamylalcohol extraction (Sambrook *et al.* 1989).

The great reed warbler has a large number of transcribed MHC class I and II genes and considerable genetic variation has been reported at these sites (Westerdahl *et al.* 1999, 2000a; Richardson & Westerdahl 2003). MHC class I exon 3 sequences (encoding the peptide-binding region of the MHC molecule) were amplified using two different sequence-specific primer combinations (HN36–GC46 and HN38–GC46). These primers amplify two different sets of sequences, that are primarily found in transcribed alleles, from one or several genes in the class I gene complex (Westerdahl *et al.* 1999, 2004). This screening method was used since it has not been feasible to develop locus-specific primers (Westerdahl 2003). The polymerase chain reaction products were separated by the denaturant gradient gel electrophoresis method according to the protocol described in Westerdahl *et al.* (2004). The screening method amplifies between two and 12 MHC class I exon 3 sequences per individual (Westerdahl 2003). An individual that has many MHC class I exon 3 sequences (hereafter called MHC bands) is likely to be heterozygous at more loci than an individual with fewer MHC bands. Hence, the number of MHC bands is used as an indicator of individual MHC heterozygosity.

Statistics

MHC similarity between females and males was calculated as MHC band-sharing; the proportion of band-sharing in a pair is twice the sum of the bands that the two individuals share divided by the sum of the bands of both individuals, i.e. $[D = 2F_{ab}/(F_a + F_b)]$ (Wetton *et al.* 1987). The power analyses were one-tailed and calculated according to Cohen (1988). All other tests used were two-tailed. The analyses were performed in SYSTAT 9.0 for Windows (Wilkinson 1998). Mean \pm SDs are reported unless otherwise stated.

Results

MHC-based female mate-choice was investigated for 279 great reed warbler breeding attempts. Each female was recorded the first time she made a breeding attempt in a season. The females had between one and 13 males available to them when making their choices. The MHC-similarity between pair mates did not differ between years, either for true pairs (ANOVA, $F_{9,269} = 0.663$, $P = 0.74$) or for random pair combinations (ANOVA, $F_{9,269} = 1.41$, $P = 0.18$; Fig. 1). There was no difference in average MHC-similarity between a female and her mate (true pair, 0.296 ± 0.145) compared with the female and a randomly possible mate (random pair, 0.287 ± 0.144 ; paired t -test, $t_{278} = 0.059$, NS). The 80% power of the former test, given our sample size ($n = 279$) and $\alpha_2 = 0.05$, should detect differences of 15% in

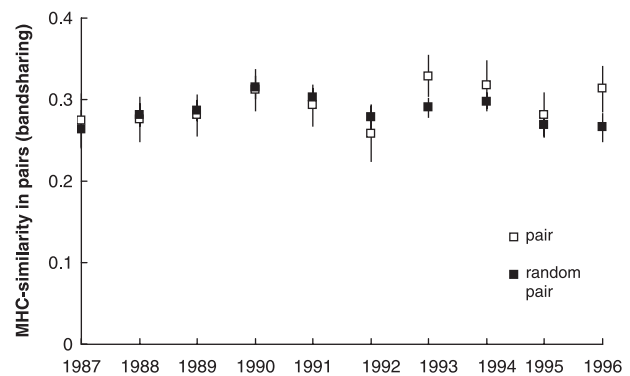


Fig. 1 Average MHC band-sharing (\pm SE), 1987–96, between the female and male in a breeding pair and the female and a randomly selected male, a male that she had the opportunity to mate with.

means between true and random pairs, hence, the data set should allow for the detection of relatively small effects. If females choose males with a certain level of MHC-similarity, true pairs should have less variance in MHC-similarity compared with random pairs. However, there was no difference in variance between true pairs ($SD = 0.145$) and random pairs ($SD = 0.144$). For each female, I tested whether the chosen male was less similar at the MHC than the average available male (paired t -test, $t_{278} = 1.01$, $P = 0.296$), however, there was no such difference.

Primary females may choose a male to receive the primary status while secondary females may be more prone to choose a mate on genetic quality. There was no difference in MHC-dependent mating preferences on female status (paired t -tests between true and random pair combinations; primary females, $t_{168} = 0.523$, $P = 0.601$; secondary females, paired t -test, $t_{109} = 0.929$, $P = 0.354$).

A high MHC allelic diversity in the offspring may be achieved if females mate with males that are heterozygous at MHC (indicated by the number of MHC bands; Fig. 2). The average number of male MHC bands did not differ between pair males (6.32 ± 1.66) and either randomly selected males (6.42 ± 1.70 ; paired t -test, $t_{278} = 0.73$, NS), or an average male (paired t -test, $t_{278} = 1.49$, $P = 0.14$). The 80% power of this test, given our sample size ($n = 279$) and $\alpha_2 = 0.05$, should detect differences of 8% in means between true and random pairs. Female status had no effect on patterns of MHC-dependent mate-choice for more heterozygous males (paired t -tests between true and random pair constellations; primary females, paired t -test, $t_{168} = 1.57$, $P = 0.12$; secondary females, paired t -test, $t_{109} = 0.38$, $P = 0.70$).

Discussion

The idea that female mate-choice in birds might be driven by genetic compatibility has been suggested several times

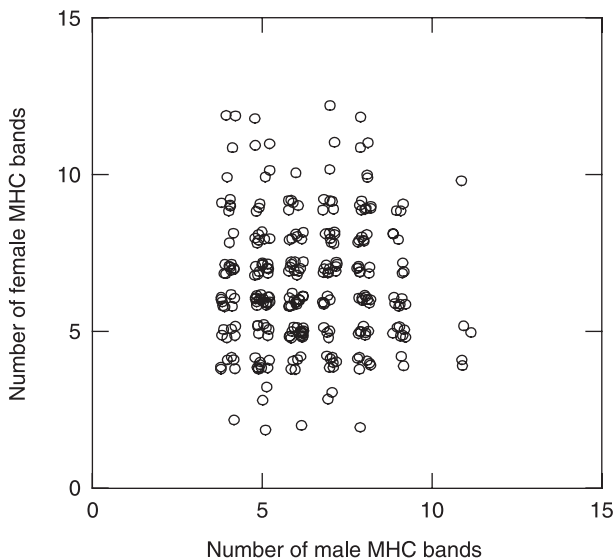


Fig. 2 Correlation between the number of female MHC-bands against the number of male MHC-bands in breeding pairs ($N = 279$).

(i.e. Johnsen *et al.* 2000; Tregenza & Wedell 2000; Blomquist *et al.* 2002). Although MHC is a likely candidate there is, to my knowledge, no study in birds reporting data on whether females choose partners based on MHC compatibility of alleles. In the present study, I investigated if there was a preference based on MHC compatibility in great reed warbler females. The large data set included 279 mating events and provided detailed information on the alternative opportunities that each female had when choosing her mate. Furthermore, I used a thorough screening method that detects differences in the DNA sequence that encodes the peptide-binding region of the MHC molecule, hence the region that is subject to balancing selection (Richardson & Westerdahl 2003; Westerdahl *et al.* 2004). Despite this, no evidence was found for great reed warbler females considering MHC compatibility when choosing their mates. A power analysis of the data set revealed that relatively small differences (15%) between true and random pairs should have been detected.

In earlier studies, it has been shown that female mate-choice in great reed warblers is associated with a number of variables (e.g. Bensch & Hasselquist 1992; Bensch 1996). For example, successful males defend territories of high quality, are older, arrive earlier and have a larger song repertoire size compared with less successful and unmated males (Hasselquist 1998). But, for the female there is on average no difference in fitness between being of primary and secondary status (Bensch 1996). There is a difference in female workload, as the female whose eggs hatch first in a territory obtains substantial help with parental care, whereas females whose clutches hatch later remain largely unassisted (Bensch 1996; Sejberg *et al.* 2000). I investigated female

mate-choice separately for primary and secondary females, as secondary females may be more prone to choose a mate on genetic quality. There was no evidence for an MHC-based mate-choice on compatibility, either for primary or secondary females. If mate-choice based on compatibility does occur in great reed warblers its effect must either be minor and/or masked by other factors that play a more important role for the female when selecting a mate.

Freeman-Gallant *et al.* (2003) recently suggested that an MHC-based mate-choice on compatibility occurs in Savannah sparrows; 1-year-old females avoided mating with MHC-similar males while older females showed no such behaviour. Surprisingly, the MHC band-sharing in Savannah sparrows was a good predictor of over-all genetic similarity between pair-mates, hence the restriction fragment length polymorphism patterns from using a single restriction enzyme and an MHC probe may not strictly estimate variation in the MHC region, but may rather be a measure of relatedness. Furthermore, Freeman-Gallant *et al.* (2003) found interesting indicative evidence that females may compensate for a genetically disadvantageous social mate-choice by gaining extra pair matings with a less related (or genetically more compatible) extra pair male based on MHC. Similar results have been reported in other bird species based on genome-wide similarity (Johnsen *et al.* 2000; Blomqvist *et al.* 2002; Foerster *et al.* 2003). In the great reed warbler, the frequency of extra pair fertilized young is low (3.1%, Hasselquist *et al.* 1995) compared with the bird species in the studies above, and should be of minor importance in terms of an MHC-based mate-choice. As expected, great reed warbler females do not have a lower MHC-similarity with the extra pair male than with the pair male (unpublished data). Extra pair matings based on MHC-similarity are probably more likely to occur in species with a higher frequency of extra pair fertilized young.

The theory of female mate-choice based on heterozygosity (Brown 1997) has been supported by a study on sticklebacks, where females preferred to mate with MHC heterozygous males (Reusch *et al.* 2001). In the great reed warbler there was no indication for an MHC-based mate-choice in favour of heterozygous males. Earlier studies of great reed warbler siblings showed that more heterozygous offspring, measured at microsatellites and MHC class I genes, are more likely to survive and return to breed (Hansson *et al.* 2001, 2004). Therefore, it would have been adaptive for females to choose males with a larger number of MHC alleles, at least for those that did not mate with a male with compatible alleles. Such mate-choice would result in at least some MHC heterozygous offspring.

In mammals and fish MHC alleles are recognized through olfactory cues both for mate-choice and kin recognition (Egid & Brown 1989; Wedekind *et al.* 1995; Olsén *et al.* 1998; Reusch *et al.* 2001). In the Soay sheep, *Ovis aries* L., there was no indication of MHC-based mate-choice

(Paterson & Pemberton 1997). One possible explanation for this may be that male–male competition determines mating in the Soay sheep, leaving limited opportunity for an active female choice in this species (Paterson & Pemberton 1997). In the great reed warbler, the female has the opportunity to choose her mate, but it is not clear whether the female can perceive the MHC genes of the different males available during the choice process. Birds have a central olfactory apparatus similar to that of other vertebrates, although *Passeriformes* are presumed to rely more on vision and hearing (Roper 1999). Female great reed warblers select their mate on the basis of the quality of his territory, the harem status she will receive, the male song repertoire size, etc. She may indirectly evaluate the MHC genes of the male, e.g. if males with specific MHC alleles are healthier, which could be indicated by superior quality. When female great reed warblers engage in extra pair matings they appear to be seeking genetic benefits for their offspring and choose males with a larger song repertoire than that of the pair male (Hasselquist *et al.* 1996). The later situation could be a scenario of female mate-choice based on a good genes effect caused by specific male alleles, and has not been investigated in the present study.

In conclusion, the great reed warbler females in a natural population at Lake Kvismaren are known to select their mate carefully (Bensch & Hasselquist 1992). Therefore, this is a mating system where a female mating preference for compatible or heterozygous MHC genes could have evolved. However, despite a large data set and the screening of highly polymorphic MHC alleles, there was no evidence of an MHC-based mate-choice.

Acknowledgements

I especially thank Staffan Bensch, Bengt Hansson, Dennis Hasselquist, Bo Nielsen and Douglas Sejberg for the brilliant fieldwork that made this study possible. I am very grateful to Staffan Bensch (S.B.), Dennis Hasselquist (D.H.), David Richardson, Torbjörn von Schantz (T.v.S.) and Håkan Wittzell for constructive comments on earlier drafts of this manuscript. This work was supported by the Swedish Research Council to S.B. and T.v.S., the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, Formas to D.H., Stiftelsen Olle Engkvist Byggmästare to S.B. and Lunds Djurskyddsfond to D.H. This is report no. 136 from Kvismare Bird Observatory.

References

- Abbas AK, Lichtman AH, Pober JS (1994) *Cellular and Molecular Immunology*. WB Saunders Company, Philadelphia.
- Andersson M (1994) *Sexual Selection*. Princeton University Press, Princeton, New Jersey.
- Bensch S (1996) Female mating status and reproductive success in the great reed warbler: is there a potential cost of polygyny that requires compensation? *Journal of Animal Ecology*, **65**, 283–296.
- Bensch S, Hasselquist D (1991) Territory infidelity in the polygynous great reed warbler *Acrocephalus arundinaceus*: the effect of variation in territory attractiveness. *Animal Ecology*, **60**, 857–871.
- Bensch S, Hasselquist D (1992) Evidence for active female mate choice in a polygynous warbler. *Animal Behavior*, **44**, 301–311.
- Bensch S, Hasselquist D (1994) Higher rate of nest loss among primary than secondary females: infanticide in the great reed warbler? *Behavioral Ecology and Sociobiology*, **35**, 309–317.
- Blomqvist D, Andersson M, Kupper C, *et al.* (2002) Genetic similarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **10**, 613–615.
- Bodmer WF (1972) Evolutionary significance of the HLA system. *Nature*, **237**, 139–145.
- Brown JL (1997) A new theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8**, 60–65.
- Carrington M, Nelson GW, Martin MP, *et al.* (1999) HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science*, **283**, 1748–1752.
- Cohen J (1988) *Statistical Power Analysis for Behavioral Sciences*. Lawrence Erlbaum, Associates Publishers, Hillsdale, NJ.
- Doherty PC, Zinkernagel RM (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, **256**, 50–52.
- Egid K, Brown JL (1989) The major histocompatibility complex and female mating preferences in mice. *Animal Behavior*, **38**, 548–550.
- Eklund AC, Belchak MM, Lapidus K, Raha-Chowdhury R, Ober C (2000) Polymorphisms in the HLA-linked olfactory receptor genes in the Hutterites. *Human Immunology*, **61**, 711–717.
- Foerster K, Delhey K, Johnsen A, Lifeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425**, 714–717.
- Freeman-Gallant CR, Meguerdichian M, Wheelwright NT, Sollecito SV (2003) Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Molecular Ecology*, **12**, 3077–3083.
- Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites. *Science*, **218**, 384–387.
- Hansson B, Bensch S, Hasselquist D, Lillandt BG, Wennerberg L, von Schantz T (2000) Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Molecular Ecology*, **9**, 1529–1538.
- Hansson B, Bensch S, Hasselquist D, Åkesson M (2001) Microsatellite diversity predicts recruitment of sibling great reed warblers. *Proceedings of the Royal Society of London B*, **268**, 1287–1291.
- Hansson B, Westerdahl H, Hasselquist D, Åkesson H, Bensch S (2004) Does linkage disequilibrium generate heterozygosity–fitness correlations in great reed warblers? *Evolution*, **58**, 870–879.
- Hasselquist D (1998) Polygyny in great reed warblers: a long term study of factors contributing to male fitness. *Ecology*, **79**, 2376–2390.
- Hasselquist D, Bensch S, von Schantz T (1995) Low frequency of extra-pair paternity in the polygynous great warbler. *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.
- Hedrick PW (2002) Pathogen resistance and genetic variation at MHC loci. *Evolution*, **56**, 1902–1908.
- Hughes AL, Hughes MK (1995) Natural selection on the peptide-binding regions of major histocompatibility complex molecules. *Immunogenetics*, **42**, 233–243.

- Johnsen A, Andersen V, Sunding C, Lifjeld JT (2000) Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature*, **406**, 296–299.
- Klein J (1986) *Natural History of the Major Histocompatibility Complex*. Wiley, New York.
- Lohm J, Grahm M, Langefors A, Andersen O, Storset A, von Schantz T (2002) Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection. *Proceedings of the R Society of London B Royal*, **269**, 2029–2033.
- Ninomiya K, Brown RE (1995) Removal of the preputial glands alters the individual odors of male MHC-congenic mice and the preferences of females for these odors. *Physiology and Behavior*, **58**, 191–194.
- Olsén KH, Grahm M, Lohm J, Langefors A (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behavior*, **56**, 319–327.
- Paterson S, Pemberton JM (1997) No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society of London B*, **264**, 1813–1819.
- Paterson S, Wilson K, Pemberton JM (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. *Proceedings of the National Academy of Sciences of the 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, Wakeland EK (1990) Evolution of diversity at the major histocompatibility complex. *Trends in Ecology and Evolution*, **5**, 181–187.
- Reusch TB, Haberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414**, 300–302.
- Richardson DS, Westerdahl H (2003) MHC diversity in two *Acrocephalus* species: the outbred Great reed warbler and the inbred Seychelles warbler. *Molecular Ecology*, **12**, 3523–3529.
- Roper TJ (1999) Olfaction in birds. *Advances in the Study of Behavior*, **28**, 247–332.
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.
- von Schantz T, Wittzell H, Göransson G, Grahm M, Persson K (1996) MHC genotype and male ornamentation: genetic evidence for the Hamilton-Zuk model. *Proceedings of the Royal Society of London B*, **263**, 265–271.
- Sejberg D, Bensch S, Hasselquist D (2000) Nestling provisioning in polygynous great reed warblers (*Acrocephalus arundinaceus*): do males bring larger prey to compensate for fewer nest visits? *Behavioral Ecology and Sociobiology*, **47**, 213–219.
- Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013–1027.
- Wedekind C, Seebeck T, Bettens F, Paepke AJ (1995) MHC-dependent mate preferences in humans. *Proceedings of the Royal Society of London B*, **260**, 245–249.
- Westerdahl H (2003) Avian MHC: variation and selection in the wild. PhD thesis Animal Ecology. Lund University, Sweden.
- Westerdahl H, Wittzell H, von Schantz T (1999) Polymorphism and transcription of MHC class I genes in a passerine bird, the great reed warbler. *Immunogenetics*, **49**, 158–170.
- Westerdahl H, Wittzell H, von Schantz T (2000a) Mhc diversity in two passerine birds: no evidence for a minimal essential MHC. *Immunogenetics*, **52**, 92–100.
- Westerdahl H, Bensch S, Hansson B, Hasselquist D (2000b) Brood sex ratios, female harem status and resources for nestling provisioning in the great reed warbler *Acrocephalus arundinaceus*. *Behavioral Ecology and Sociobiology*, **47**, 312–318.
- 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 JH, Carter RE, Parkin DT, Walters D (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*, **327**, 147–149.
- Wilkinson L (1998) *SYSTAT 9.0 for Windows*. SPSS Inc., Evanston, IL.
- Zelano B, Edwards SV (2002) An MHC component to kin recognition and mate choice in birds: Predictions, progress, and prospects. *American Naturalist*, **160**, S225–237.