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ORIGINAL PAPER

Male transmission ratio distortion supports MHC-linked cryptic female choice in the lesser kestrel (Aves: Falconidae)

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Abstract Major histocompatibility complex (MHC) genes are classical examples of "good genes" because of their critical participation in fighting and eradicating pathogens. Here, we investigated the inheritance of alleles across a highly polymorphic MHC class II B gene in a socially monogamous raptor, the lesser kestrel *Falco naumanni*. Allele segregation patterns from parents to offspring were monitored in 44 breeding pairs and 228 nestlings. We found that a particular array of closely related alleles, defined by the presence of two of the three commonest alleles (only three alleles exhibit frequencies larger than 10 % in the studied populations), showed significant transmission ratio distortions when inherited from males (k=0.702±0.188, p<0.001), but not when inherited from females (k=0.563±0.207, p=0.19). We believe that this result is consistent with the targeting of genetically loaded spermatozoa by female kestrels. Our analyses do not allow discerning, however, whether this pattern is predominantly driven by sperm competition or differential maternal allocation. This trend, nonetheless, would agree with the contrasting relative frequencies of these alleles between western and eastern populations of lesser kestrels and sustains their contemplation as locally adapted alleles, as suggested in previous studies. Ours is one of the very few studies supporting the MHC-based female cryptic choice in birds and opens inspiring lines of research. In particular, future studies addressing the influence of candidate MHC alleles on individual fitness and their effect on maternal adjustments associated with reproduction should be encouraged.

Keywords Immunogenetics · Lesser kestrels · MHC · Post-copulatory selection · Sperm competition

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Introduction

Major histocompatibility complex (MHC) hosts a series of genes that plays a critical role in battling pathogen and parasite infections (reviewed by Sommer 2005; Piertney and Oliver 2006; Spurgin and Richardson 2010). MHC genes encode for glycoproteins that display antigens from both intra- and extracellular pathogens on the surface of specialized cells of the immune system. The capability of a unique MHC molecule to bind and present antigens is limited but critical to trigger the adaptive branch of the immune response. Here, T-cell activation is responsible for the removal of pathogen-infected cells and the proliferation of antibody-producing plasma and memory B cells. The spectrum of pathogens a particular individual can respond to is in some part linked to its degree of MHC polymorphism. It is therefore not surprising that evolutionary mechanisms such as balancing selection, either through heterozygote advantage or frequency-dependent selection, gene duplication and inter- or intragenic recombination contribute to the maintenance of extraordinary high levels of MHC polymorphism within and between populations (Richman et al. 2003; Sommer 2005; Piertney and Oliver

2006; Bollmer et al. 2010; but see Radwan et al. (2010); Gangoso et al. 2012).

Mating preferences might also have a profound effect on MHC diversity (Zelano and Edwards 2002; Havlicek and Roberts 2009). MHC genes are believed to play a critical role in self versus non-self recognition and have been suggested to influence individual odours (reviewed by Ziegler et al. 2010). For these reasons, MHC molecules have been widely related to both pre- and post-copulatory mating decisions. For instance, individuals might assess their partner's MHC genotype through emitted scents and then choose among the most dissimilar individuals in order to maximize offspring fitness. This constitutes the baseline for pre-copulatory MHC-disassortative mating preferences. However, this theory is currently believed to be overly simplistic as it does not consider the targeting of selectively advantageous alleles (MHC genes are classical examples of "good" genes that can influence indicators of individual quality, e.g. Olsson et al. 2005; Baratti et al. 2010), the putative detrimental effects of outbreeding depression or incompatibility between alleles and the promotion of an optimal, intermediate number of alleles and allele divergence (e.g. Roberts 2009; Lenz et al. 2009). Furthermore, several empirical studies have failed to report any sign of pre-copulatory MHC-disassortative mating preferences in different taxa, hence questioning its generality in vertebrates (e.g. Westerdahl 2004; Ekblom et al. 2004; Derti et al. 2010).

Female cryptic choice is also believed to be important in shaping MHC variation. According to the "Sperm Receptor Selection hypothesis" formulated by Ziegler et al. (2002), females might actively select over genetically loaded spermatozoa (see also, for instance, Pryke et al. (2010)). Although MHC molecules do not seem to be expressed in the surface of gametes, at least in humans, odour receptor genes in strong linkage disequilibrium with MHC genes would denote in some way the MHC alleles carried by particular sperm cells (see review by Ziegler et al. (2010)). It has been then suggested that females would exert stronger attraction for dissimilar MHC molecules regarding their own within their reproductive tracts (see Ziegler et al. (2010)). The MHC is also believed to influence maternal–foetal interactions in viviparous species such as mammals, for instance, regarding the selective abortion of MHC homozygous embryos (e.g. Ober et al. 1993; Knapp et al. 1996). In oviparous species such as birds, the degree of contact between the mother and the developing embryo is more limited because of the premature formation of the eggshell. However, it is believed that females can allocate more energetic resources for genetically optimal offspring (e.g. Pryke and Griffith 2009, 2010), although the molecular pathway whereby this occurs remains obscure.

In this study, we have investigated the inheritance of MHC class II alleles (exon 2, β chain) in a small bird of prey, the lesser kestrel *Falco naumanni*. This small and socially monogamous falcon represents a very suitable species for the investigation of MHC-dependent post-copulatory selection because of the possibility to survey highly polymorphic (>100 alleles) and single-copy MHC class II genes (Alcaide et al. 2008) across a large number of individuals with known kinship relationships. Previous research on this species has reported strong patterns of MHC genetic structuring across its breeding range (Alcaide et al. 2008). In fact, the presence or absence of certain alleles assisted the elucidation of the wintering quarters of this migratory bird in sub-Saharan Africa (Rodríguez et al. 2011). Average heterozygosity values at this locus exceeded 0.90, and just a few alleles showed frequencies larger than 10 %. In Western Europe, only three closely related alleles (Fana1, Fana2 and Fana3) and the divergent allele, Fana19, showed frequencies larger than 10 % in different populations (see Alcaide et al. (2008); Table 1). On the one hand, it can be argued that common alleles are the most efficient to counteract local pathogen communities. On the other hand, pathogens could have evolved mechanisms to avoid being detected by the most extended host defences, and therefore, common alleles would be selected against. Under this scenario, we aimed to find support for one of the following hypotheses: (1) common and locally adapted MHC alleles are selectively advantageous, and therefore, there must exist a cryptic female choice for these alleles; (2) common MHC alleles are selectively disadvantageous because pathogens have evolved resistance, and consequently, there must be a cryptic female choice against these alleles; and (3) there are

Table 1 Frequency of the most common MHC class II B alleles (exon 2) of the lesser kestrel in the Iberian Peninsula

Allele name	GenBank account number	Frequency
Fana2 (A)	EF370840	0.16
Fana19 (B)	EF370854	0.128
Fana1 (A)	EF370839	0.106
Fana36	EF370861	0.066
Fana3 (A)	EF370841	0.025
Fana8	EF370851	0.022
Fana12	EF370849	0.022
Fana62 (B)	EU107741	0.018
Fana10	EF370842	0.018
Fana83	EF370857	0.018
Fana40	EU107734	0.018
Fana29	EF370860	0.015
Fana82	EF370856	0.015

This table was built in accordance with the lesser kestrels that were genotyped in the study of Alcaide et al. (2008) and the adult wild birds genotyped in the present study. Whether the alleles belong to the supertype A or B is also indicated in brackets

no significant differences between the observed and expected frequencies of common alleles in the offspring, thus suggesting a lack of post-copulatory selection at the kestrel MHC class II.

Materials and methods

Sampling of lesser kestrel families

We gathered DNA samples from adults and between four and ten nestlings (5.18±1.61 nestlings, on average, per breeding pair) in 44 families of lesser kestrels (see Table S1 of the "Electronic supplementary material"). We monitored 228 meiotic events, and the minimum brood size of four nestlings allows at least one statistical chance for every combination of alleles derived from a biparental and diploid genetic system (i.e. 1/4 probability for each allele combination). Blood samples and feathers from adult kestrels and their offspring were collected, and DNA was extracted as described in Alcaide et al. (2009). Twenty-four of the breeding pairs analyzed here belonged to a captive breeding centre (DEMA, www. demaprimilla.org) sited in Extremadura (Spain). Captive kestrels were kept in colonial enclosures that accommodated several breeding pairs. Laid eggs are immediately removed through a small compartment connected to each nest that is accessible from the outside of the colonial enclosure, and hence, this practice causes no disturbance at all to the captive colony. Eggs are artificially incubated, and once hatched, newborns are fed using irrecoverable individuals and then released during ongoing reintroduction programmes of the species. Kinship relationships within these captive breeding enclosures have been previously resolved using microsatellites as described in Alcaide et al. (2009). Twenty additional families were sampled from wild colonies located in north-eastern and south-western Spain. Kinship relationships for about one half of these families were resolved in a previous study also based on microsatellite variation (see Alcaide et al. (2005)). For the rest of the sampled families, we were confident that the attending pairs of the studied nests were the true parents of the nestlings sampled. In this regard, we appeal to the low rates of extra-pair paternity and intra-specific brood parasitism documented in this colonially breeding species (Alcaide et al. 2005). Moreover, we were confident that the high polymorphism of the kestrel MHC class II would uncover any potential mismatch between paternal and offspring genotypes, and consequently, problematic families could be discarded.

Genetic and statistical analyses

The entire exon 2 of a single MHC class II B gene was PCR-amplified and directly sequenced using primers Fal2FC and Fal2RC (Alcaide et al. 2007). Individuals are likely to be heterozygous at this locus. Consequently, the gametic phase of each individual was resolved by a combination of computational inferences based on the PHASE algorithm run in DnaSP 5.0 (Librado and Rozas 2009; see also Alcaide et al. (2011) for a direct application on lesser kestrel MHC genes) and direct observations of allele segregation patterns from parents to offspring. We investigated the transmission rates of the common alleles Fana1 and Fana2 and other alleles not different from these alleles in more than two amino acid positions. This criterion permitted us to include the alleles Fana3, Fana4, Fana23 and Fana111. This group of alleles was thereafter referred as allele supertype A. Likewise, we investigated the segregation of the common allele Fana19 and the closely related Fana62 and Fana7 alleles, thereafter referred as allele supertype B (see Table 1). Certainly, MHC alleles have been usually classified into MHC supertypes in accordance with their expected antigen-binding capabilities (e.g. Schwensow et al. 2008), and related alleles are expected to bind similar antigens. Clustering alleles into MHC supertypes also allows us to include more cases in our analyses. This is indeed a critical issue because of the huge genetic polymorphism of the locus under investigation. The extreme genetic polymorphism and allele divergence of the kestrel MHC class II B locus (>100 alleles and >20 nucleotide differences, on average, among alleles) would nonetheless be consistent with the classification of alleles into MHC supertypes (see Fig. 1; Alcaide et al. 2008). In addition, it should be noted that mean allele divergence within each of the proposed allele supertypes is

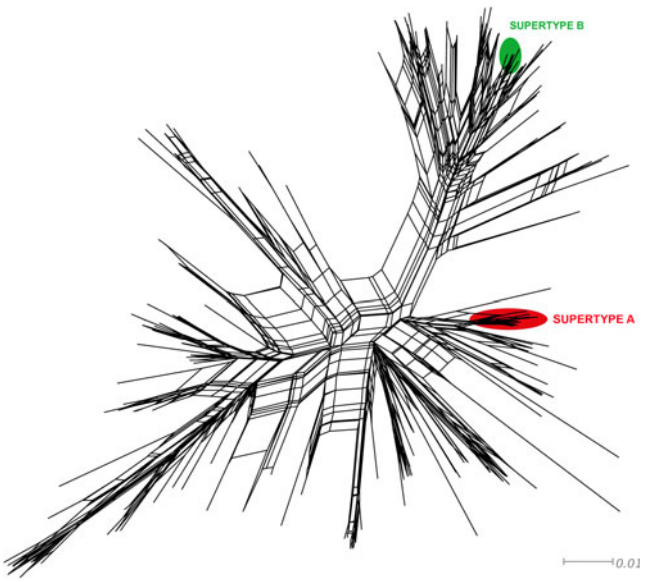

Fig. 1 The Neighbor-Net network of more than 100 MHC class II B alleles (exon 2) isolated in the lesser kestrel built in SplitsTree 4.0 (Huson and Bryant 2006). Note that not all the alleles were found in the breeding pairs from the present study (see also Alcaide et al. (2008)). The location of the two allele supertypes investigated here is indicated (supertype A, in *red*; supertype B, in *green*)

lower than that obtained when comparing any of the supertype A or B alleles with other kestrel MHC alleles (see coloured clusters in Fig. 1).

Deviations from expected the Mendelian segregation ratios of candidate alleles were investigated. Under the null hypothesis of no departure from Mendel's rule, the transmission rate (k) of a particular allele supertype, averaged across individuals, must not significantly differ from parity (i.e. 1/2). The calculation of the transmission rate for candidate alleles is very straightforward, and it is based on the proportion of nestlings that inherited a particular allele from a heterozygous parent (see, for instance, Aparicio et al. (2010)). As an example, in a cross Aa (male genotype) × AA (female genotype) that generates four nestlings, the transmission rate of the male "a" allele would be k_a=1 if the four nestlings are Aa, k_a=0.5 (expected Mendelian segregation ratio) if two out of the four nestlings are Aa and k_a=0 if the four nestlings are AA. We carried out two different analyses: one for the alleles belonging to the supertype A and another for the alleles belonging to the supertype B. Note that we only included in the present study those breeding pairs where at least one of the adult birds was heterozygous and included one supertype A or supertype B allele along with other alleles.

Results

The transmission rate of supertype A alleles, averaged across individuals, was significantly different from parity when inherited from adult males (k=0.702±0.188, t=4.93, df=20, p<0.001, N=21 families and 111 meiotic events). This trend was observed independently for both captive (k=0.634±0.161, t=−2,88, df=11, p=0.014, N=12 families and 73 meiotic events) and wild males (k=0.794±0.191, t=4.62, df=8, p=0.0017, N=9 families and 38 meiotic events), although the transmission rate for wild males is almost significantly higher than that for captive males (t=−2,085, df=19, p=0.051). On the contrary, we did not find significant deviations from expected Mendelian segregation ratios when monitoring the segregation of supertype A alleles inherited from adult females (k=0.563±0.207, t=1.34, df=18, p=0.19, N=19 families and 91 meiotic events). The transmission rate of supertype A alleles inherited from males was also significantly higher than that observed from females (t=2.22, df=38, p=0.032). Interestingly, supertype A alleles co-segregated together more frequently than expected (Wilcoxon signed-rank test, Z=2.55, p=0.01, N=12 families) even under the possibility of alternative genotypic configurations formed by two divergent alleles. For allele supertype B, transmission rates did not significantly differ from parity either in males (k=0.506±0.196, t=0.075, df=7, p=0.93, N=10 families and 40 meiotic events) or in females (k=0.494±0.064, t=−0.087, df=15, p=0.93, N=16 families and 76 meiotic events). However, we found that Fana1 and Fana2 alleles co-segregated with Fana19 more frequently than expected under random allele segregation (Wilcoxon signed-rank test, Z=2.49, p=0.012, N=16 families). Paternal and offspring genotypes for the different families are displayed in Table S1 of the "Electronic supplementary material".

Discussion

This study reports significant deviations from expected Mendelian segregation ratios for a common MHC class II supertype in Western Europe when inherited from male lesser kestrels, but not when inherited from females. This phenomenon, known as male transmission ratio distortion (see for instance LeMaire-Adkins and Hunt (2000)), seems to more decisively affect wild colonies than captive colonies. Perhaps, selective regimes in captive colonial enclosures with ad libitum feeding and artificial incubation of eggs (see Alcaide et al. (2009)) are more relaxed than in natural conditions. Nevertheless, our sample size is not high enough to reach any strong conclusion in this respect. Overall, the transmission rate of supertype A alleles in males (k=0.70) exceeds the highest found across 53 presumably neutral microsatellite alleles (k=0.62) in a former study on the same species (Aparicio et al. 2010). Previously, Aparicio et al. (2010) only found subtle deviations from the Mendelian proportions in two microsatellite alleles: one in males and the other in females. MHC genes are certainly more prone to be affected by selective processes than presumably neutral microsatellites. Two plausible mechanisms could drive post-copulatory selection of MHC alleles in the female oviduct: differential chemo-attraction of genetically loaded spermatozoa and/or differential embryo outcome (see review by Ziegler et al. (2010)). That said, we cannot rule out the contribution of other distortive processes occurring during the production of male sperm cells (e.g. LeMaire-Adkins and Hunt 2000).

The sperm receptor hypothesis (reviewed by Ziegler et al. 2010) is applicable to avian species given that birds are known to have numerous functional odour receptor genes (e.g Steiger et al. 2008) that could play an important role in self versus non-self recognition. Several studies in songbirds have indeed shown that females from socially monogamous species are more prone to engage in extra-pair fertilizations when paired to similar MHC or males with low MHC diversity (e.g. Freeman-Gallant et al. 2003; Richardson et al. 2005; Promerová et al. 2011). A recent study conducted in the Seychelles warbler *Acrocephalus sechellensis* even demonstrated hidden benefits in offspring derived from female infidelity (Brouwer et al. 2010). However, no study to date has clearly shown whether the targeting of MHC

genes is driven by pre- or post-copulatory mechanisms in birds (but see evidence of microsatellite-based post-copulatory selection against inbreeding in Brekke et al. (2011)). A recent study in a fish, the guppy *Poecilia reticulata*, has unravelled an important role of sperm competition mediated by the interaction between the sperm and the ovarian fluid, with gametes for unrelated males being more prone to fertilize the eggs (Gasparini and Pilastro 2011). At first sight, our results are contradictory with the idea that females might preferentially target male gametes with dissimilar MHC haplotypes as theirs. We found, on the contrary, that homozygous birds for the supertype A are more frequently found in the offspring than expected. This result therefore suggests that females might be more strongly targeting particular alleles rather than betting for MHC dissimilarity (see also, for instance, Roberts (2009)). Increased fertilization success for particular MHC alleles has been demonstrated, for instance, in the Arctic char *Salvelinus alpinus* (Skarstein et al. 2005). Nevertheless, the molecular mechanisms, through which spermatozoa carrying particular alleles are more prone to fertilize the egg, still remain unclear.

In accordance with the differential allocation hypothesis (Burley 1986; Sheldon 2000), it is also possible that females develop breeding strategies based on the investment of more energetic resources into those eggs fertilized by spermatozoa carrying certain genes. The embryos developing within those eggs could thus have higher chances to hatch and survive as newborns. Indeed, recent studies in gouldian finches *Erythrura gouldiae* have stressed out the importance of maternal adjustments during reproduction. Females, but not males, increased their provisioning effort when paired with compatible mates. Clutch, egg size and care to offspring decreased when females were forcedly paired with incompatible males, and females were more prone to overproduce male offspring (Pryke and Griffith 2009, 2010). In our case of interest, it would be possible that female kestrels differentially allocate energetic resources in order to promote immunological optimum offspring. It is important to mention here that the immunological properties of individuals cannot, of course, be exclusively inferred by a single locus. MHC genes are, however, embedded into large haplotypic blocks in strong linkage disequilibrium. Therefore, MHC alleles are likely to co-segregate with other functionally important and co-evolving immune genes (e.g. Walsh et al. 2003).

Our findings would be also in agreement with the positive frequency-dependent selection, at least at the MHC supertype level. Previously, Aparicio et al. (2010) also reported a positive correlation between the transmission rates of certain microsatellite alleles and their frequencies in the population. Theory predicts, on the other hand, that common alleles might have a selective disadvantage because pathogens can rapidly change their antigenicity and thus evade recognition by the most abundant host alleles in the population (e.g. "Red Queen hypothesis", Ladle 1992). In the absence of any temporal comparison, it is certainly difficult to anticipate whether supertype A alleles were formerly present at low frequencies in the population and/or whether selective regimes have strongly turned around in the population. The genotyping of museum specimens would shed important light in this respect. In addition, we cannot rule out that emerging selectively advantageous alleles just incorporate a few modifications in their amino acid sequence rather than radical shifts in the type of the alleles which are being selected for, and in some way, particular MHC supertypes can thus endure during long periods of time. In support of this view, we observed that small variants within the supertype A (e.g. Fana4, Fana23 and Fana111 alleles) were more frequently inherited in the offspring (k=0.64, N=26 meiotic events) than the commonest Fana2 allele (see family ID numbers 3, 6 and 21, Table S1 of the "Electronic supplementary material"). Conversely, the Fana2 allele was preferentially transmitted from males to the offspring when in heterozygosis with other divergent alleles (k=0.63, family ID numbers 2, 10, 12, 23, 31, 32 and 43, N=35 meiotic events). Although extremely interesting, the low number of males carrying these alleles in our study system impedes to reach any strong conclusion. The harness of adequate statistical support is certainly challenging given the extreme polymorphism of the locus under investigation and the very low frequencies of the vast majority of alleles. Finally, even though we did not detect any evidence of post-copulatory selection favouring heterozygous offspring, we cannot rule out that heterozygosity may indeed play an important role during the life of an individual. This potential heterozygous advantage, together with stochastic events, could therefore buffer in some way a sharp increase of certain allele supertypes during future generations.

Conclusion

This study provides empirical evidence supporting the cryptic female choice in lesser kestrels. Our results suggest that females might indeed target genetically loaded spermatozoa (see also Pryke et al. (2010)), but we cannot discern whether this process is driven by sperm competition in the female oviduct or by differential maternal allocation. Interestingly, the alleles presumably targeted by females belong to an MHC supertype that is very abundant in the Western distribution range of the species but very rare in the Eastern distribution range (see Alcaide et al. (2008)). This finding therefore supports the idea that these alleles might be adapted to counteract local pathogen communities. Motivating and fruitful lines of research may thus arise from the present study. First, future studies must investigate the relationship

between these candidate alleles and individual fitness components in the studied population. Second, research about putative maternal adjustments must be encouraged. For instance, the differential allocation hypothesis would find a strong support if the eggs fertilized by supertype A alleles are bigger and if nestlings hatch earlier and are in better condition than eggs fertilized by spermatozoa carrying alternative alleles. Likewise, hatching success could be related with the presence of presumably advantageous alleles in the paternal genotypes.

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Ethical standards We hereby declare that the present study complies with the ethical standards of animal research in Spain.

References

Alcaide M, Negro JJ, Serrano D, Tella JL, Rodríguez C (2005) Extra-pair paternity in the lesser kestrel *Falco naumanni*: a re-evaluation using microsatellite. IBIS 147:608–611

Alcaide M, Edwards SV, Negro JJ (2007) Characterization, polymorphism, and evolution of MHC class II B genes in birds of prey. J Mol Evol 65:541–54

Alcaide M, Edwards SV, Negro JJ, Serrano D, Tella JL (2008) Extensive polymorphism and geographical variation at a positively selected MHC class II B gene of the lesser kestrel (*Falco naumanni*). Mol Ecol 17:2652–2665

Alcaide M, Negro JJ, Serrano D, Antolín JL, Casado S, Pomarol M (2009) Captive breeding and reintroduction of the lesser kestrel *Falco naumanni*: a genetic analysis using microsatellites. Conserv Genet 11:331–338

Alcaide M, Rodríguez A, Negro JJ (2011) Sampling strategies for accurate computational inferences of gametic phase across highly polymorphic major histocompatibility complex loci. BMC Res Notes 4:151

Aparicio JM, Ortego J, Calabuig G, Cordero PJ (2010) Evidence of subtle departures from Mendelian segregation in a wild lesser kestrel (*Falco naumanni*) population. Hered 105:213–9

Baratti M, Ammannati M, Magnelli C, Massolo A, Dessì-Fulgheri F (2010) Are large wattles related to particular MHC genotypes in the male pheasant? Genetica 138:657–65

Bollmer JL, Dunn PO, Whittingham LA, Wimpee C (2010) Extensive MHC class II B gene duplication in a passerine, the common yellowthroat (*Geothlypis trichas*). J Hered 101:448–60

Brekke P, Wang J, Bennett PM, Cassey P, Dawson DA, Horsburgh GJ, Ewen JG (2011) Post-copulatory mechanisms of inbreeding avoidance in the island endemic hihi (*Notiomystis cincta*). Behav Ecol 23:278–284

Brouwer L, Barr I, van de Pol M, Burke T, Komdeur J, Richardson DS (2010) MHC-dependent survival in a wild population: evidence for hidden genetic benefits gained through extra-pair fertilizations. Mol Ecol 19:3444–55

Burley N (1986) Sexual selection for aesthetic traits in species with biparental care. Am Nat 127:415–455

Derti A, Cenik C, Kraft P, Roth FP (2010) Absence of evidence for MHC-dependent mate selection within HapMap populations. PLoS Genet 6:e1000925

Ekblom R, Saether SA, Grahn M, Fiske P, Kålås JA, Höglund J (2004) Major histocompatibility complex variation and mate choice in a lekking bird, the great snipe (*Gallinago media*). Mol Ecol 13:3821–8

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. Mol Ecol 12:3077–3083

Gangoso L, Alcaide M, Grande JM, Muñoz J, Talbot SL, Sonsthagen SA, Sage KA, Figuerola J (2012) Colonizing the world in spite of reduced MHC variation. J Evol Biol 25:1438–1447

Gasparini C, Pilastro A (2011) Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. Proc R Soc Lond B 278:2495–501

Havlicek J, Roberts SC (2009) MHC-correlated mate choice in humans: a review. Psychoneuroendocrinology 34:497–512

Huson D, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23:254–267

Knapp LA, Hab JC, Sackettb GP (1996) Reproductive parental MHC antigen sharing and pregnancy wastage in captive pigtailed macaques. Immunology 32:73–88

Ladle RJ (1992) Parasites and sex: catching the red queen. Trends Ecol Evol 7:405–8

LeMaire-Adkins R, Hunt PA (2000) Non-random segregation of the mouse univalent X chromosome: evidence of spindle-mediated meiotic drive. Genetics 156:775–83

Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M (2009) Disentangling the role of MHC-dependent "good genes" and "compatible genes" in mate-choice decisions of three-spined sticklebacks *Gasterosteus aculeatus* under semi-natural conditions. J Fish Biol 75:2122–42

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Ober C, Steck T, van der Ven K, Billstrand C, Messer L, Kwak J, Beaman K, Beer A (1993) MHC class II compatibility in aborted fetuses and term infants of couples with recurrent spontaneous abortion. J Reprod Immunol 25:195–207

Olsson M, Madsen T, Wapstra E, Silverin B, Ujvari B, Wittzell H (2005) MHC, health, color, and reproductive success in sand lizards. Behav Ecol Sociobiol 58:289–294

Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. Hered 96:7–21

Promerová M, Vinkler M, Bryja J, Poláková R, Schnitzer J, Munclinger P, Albrecht T (2011) Occurrence of extra-pair paternity is connected to social male's MHC-variability in the scarlet rosefinch *Carpodacus erythrinus*. J Avian Biol 42:5–10

Pryke SR, Griffith SC (2009) Genetic incompatibility drives sex allocation and maternal investment in a polymorphic finch. Science 323:1605–7

Pryke SR, Griffith SC (2010) Maternal adjustment of parental effort in relation to mate compatibility affects offspring development. Behav Ecol 21:226–232

Pryke SR, Rollins LA, Griffith SC (2010) Females use multiple mating and genetically loaded sperm competition to target compatible genes. Science 329:964–7

Radwan J, Biedrzycka A, Babik W (2010) Does reduced MHC diversity decrease viability of vertebrate populations? Biol Conserv 143:537–544

Richardson DS, Komdeur J, Burke T, von Schantz T (2005) MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. Proc R Soc Lond B 272:759–67

Richman AD, Herrera LG, Nash D, Schierup MH (2003) Relative roles of mutation and recombination in generating allelic polymorphism at an MHC class II locus in *Peromyscus maniculatus*. Genet Res 82:89–99

Roberts SC (2009) Complexity and context of MHC-correlated mating preferences in wild populations. Mol Ecol 18:3121–3

Rodríguez A, Alcaide M, Negro JJ, Pilard P (2011) Using major histocompatibility complex markers to assign the geographic origin of migratory birds: examples from the threatened lesser kestrel. Anim Conserv 14:306–313

Schwensow N, Eberle M, Sommer S (2008) Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. Proc R Soc Lond B 275:555–64

Sheldon B (2000) Differential allocation: tests, mechanisms and implications. Trends Ecol Evol 15:397–402

Skarstein F, Folstad I, Liljedal S, Grahn M (2005) MHC and fertilization success in the Arctic charr (*Salvelinus alpinus*). Behav Ecol Sociobiol 57:374–380

Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Front Zool 2:16

Spurgin LG, Richardson DS (2010) How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proc R Soc Lond B 277:979–88

Steiger SS, Fidler AE, Valcu M, Kempenaers B (2008) Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? Proc R Soc Lond B 275:2309–17

Walsh EC, Mather KA, Schaffner SF, Farwell L, Daly MJ, Patterson N, Cullen M, Carrington M, Bugawan TL, Erlich H et al (2003) An integrated haplotype map of the human major histocompatibility complex. Am J Hum Genet 73:580–90

Westerdahl H (2004) No evidence of an MHC-based female mating preference in great reed warblers. Mol Ecol 13:2465–70

Zelano B, Edwards SV (2002) An MHC component to kin recognition and mate choice in birds: predictions, progress, and prospects. Am Nat 160:S225–37

Ziegler A, Dohr G, Uchanska-Ziegler B (2002) Possible roles for products of polymorphic MHC and linked olfactory receptor genes during selection processes in reproduction. Am J Reprod Immunol 48:34–42

Ziegler A, Santos PSC, Kellermann T, Uchanska-Ziegler B (2010) Self/non-self perception, reproduction and the extended MHC. Self/Non-self 1:176–191