Cryptic preference for MHC-dissimilar females in male red junglefowl, *Gallus gallus*

Mark A. F. Gillingham¹, David S. Richardson², Hanne Løvlie¹,
Anna Moynihan^{1,3}, Kirsty Worley^{1,2} and Tom Pizzari^{1,*}

¹Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, OX1 3PS, UK

²School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

³School of Biological Sciences, Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3JT, UK

An increasing number of studies test the idea that females increase offspring fitness by biasing fertilization in favour of genetically compatible partners; however, few have investigated or controlled for corresponding preferences in males. Here, we experimentally test whether male red junglefowl, *Gallus gallus*, prefer genetically compatible females, measured by similarity at the major histocompatibility complex (MHC), a key gene complex in vertebrate immune function. Theory predicts that because some degree of MHC heterozygosity favours viability, individuals should prefer partners that carry MHC alleles different from their own. While male fowl showed no preference when simultaneously presented with an MHC-similar and an MHC-dissimilar female, they showed a ‘cryptic’ preference, by allocating more sperm to the most MHC-dissimilar of two sequentially presented females. These results provide the first experimental evidence that males might respond to the MHC similarity of a female through differential ejaculate expenditure. By revealing that cryptic male behaviours may bias fertilization success in favour of genetically compatible partners, this study demonstrates the need to experimentally disentangle male and female effects when studying preferences for genetically compatible partners.

Keywords: major histocompatibility complex; sexual selection; mate choice; genetic compatibility; sperm allocation; kin discrimination

1. INTRODUCTION

An enduring challenge in evolutionary biology is to elucidate the mechanisms that maintain polymorphism at fitness-related loci in the face of Darwinian selection. One such potential mechanism that has attracted increasing interest is mate preference based on the genetic complementarity of maternal and paternal genotypes, which is expected to evolve as a response to a number of selective advantages associated with, or conveyed by, offspring heterozygosity (Zeh & Zeh 1997; Tregenza & Wedell 2000; Colegrave *et al.* 2002; Neff & Pitcher 2005; Kempenaers 2007). Because higher gametic investment is thought to result ultimately in more pronounced choosiness, most studies have explored this idea focusing on female mating preferences. However, evidence that females bias mating success in favour of genetically compatible males, or sperm utilisation in favour of their ejaculates, is often equivocal. While some bias in mating or fertilization success have sometimes been interpreted as evidence of female preference for genetically compatible males (e.g. Olsson *et al.* 1996; Bretman *et al.* 2004; Thuman & Griffith 2005; Simmons *et al.* 2006; Hoffman *et al.* 2007; Jehle *et al.* 2007), other studies have failed to detect such effects (e.g. Jennions *et al.* 2004). The potential for genetic compatibility to explain the maintenance of additive variation in fitness traits therefore remains debated (Kotiaho *et al.* 2008).

An all too often neglected issue in investigations of female preference for genetically compatible males is the

possibility that males too might bias their mating effort based on the genetic compatibility of a female. There is mounting evidence that males preferentially invest in certain females, ‘overtly’ through mating preferences (e.g. Trivers 1972; Burley 1977; Parker 1983; Jones & Hunter 1993; Owens & Thompson 1994; Johnstone *et al.* 1996; Amundsen & Forsgren 2001; Bonduriansky 2001; Kokko & Monaghan 2001; Byrne & Rice 2006; Chenoweth *et al.* 2006, 2007) and ‘cryptically’ through preferential ejaculate expenditure (Bonduriansky 2001; Reinhold *et al.* 2002; Wedell *et al.* 2002; Pizzari *et al.* 2003; Cornwallis & Birkhead 2006; Rubolini *et al.* 2006), particularly in species where sperm competition favours strategic sperm allocation (Wedell *et al.* 2002). It is well established that the relative number of sperm inseminated by competing males into a female is a reliable predictor of fertilization success in several species (e.g. Gage & Morrow 2003), including the fowl (Martin *et al.* 1974). Cryptic male preferences through differential male sperm investment in certain females may therefore influence fertilization success and represent a neglected source of variation in paternity.

Little is known about male mating preferences mediated by genetic compatibility. However, we do know that because of anisogamy, male preference functions may diverge from female preference functions: under some circumstances, males are selected to invest in genetically incompatible females while females are selected to resist fertilizations by genetically incompatible males, leading to sex-specific counteracting responses (Parker 1979; Ball & Parker 2003; Pizzari *et al.* 2004;

* Author for correspondence (tommaso.pizzari@zoo.ox.ac.uk).

Kokko & Ots 2006; Parker 2006). In addition, we would expect male mating bias to be modulated by female availability, being more pronounced when different females are simultaneously, rather than sequentially, available to a male (Kokko & Ots 2006). Taken together, these factors indicate that the patterns of male preference can be both subtle and complex.

Studying male preferences for genetically compatible females is key to our understanding of selection on—and polymorphism of—fitness-related loci for two reasons. First, male preferences can potentially confound the study of biases in mating and fertilization success attributed to female preferences. For example, a bias in favour of genetically compatible fertilizations can be due to female preference, male preference or a combination of both. Similarly, lack of a fertilization bias may reflect lack of mating preference in both sexes or, alternatively, the outcome of counteracting strategies. Second, intersexual selection driven by male mating preferences is a neglected episode of sexual selection, and elucidating the evolution of male preferences is an important challenge in sexual selection theory (Wright *et al.* 2007; Pizzari & Bonduriansky 2009).

Here, we study overt and cryptic male preference for genetically compatible partners focusing on one of the best characterized candidates of genetic compatibility, the vertebrate major histocompatibility complex (MHC). MHC genes code for antigen-presenting molecules that are important in the acquired immune system (Janeway *et al.* 1999; Glick 2000). To some extent, more MHC diverse individuals may be more likely to resist a larger variety of parasites and pathogens than less MHC diverse individuals, either because of heterozygote advantage or because of increased likelihood of carrying MHC alleles that have a greater affinity for a particular pathogen (Penn & Potts 1999; Milinski 2006). A preference for MHC-dissimilar partners will maximize the MHC diversity of offspring and, therefore, improve their ability to cope with pathogens. Furthermore, because balancing selection maintains extreme MHC polymorphism, individuals sharing MHC alleles are likely to be genetically related (Brown 1983; Potts & Wakeland 1993; Brown & Eklund 1994; Penn & Potts 1999). Therefore, independent of pathogen resistance, preference for MHC-dissimilar partners may also mediate inbreeding avoidance (Brown 1983; Potts & Wakeland 1993; Brown & Eklund 1994; Penn & Potts 1999). Either way, by preferentially investing in MHC-dissimilar partners, individuals may increase offspring fitness.

While several studies have explored female preference for MHC-dissimilar males (e.g. Wedekind & Furi 1997; Olsson *et al.* 2003; Richardson *et al.* 2005; Schwensow *et al.* 2008), information on MHC-mediated mate choice in males is scarce and mostly limited to inbred lines of mice, in which evidence for male preference for MHC-dissimilar partners has been found by some (Yamazaki *et al.* 1976, 1978, 1988; Beauchamp *et al.* 1988), but not other studies (Eklund *et al.* 1991). In addition, there is some evidence that men preferred the odour of MHC-dissimilar women (Wedekind & Furi 1997). Male differential ejaculate expenditure according to the MHC similarity of a male with a female has not—to our knowledge—been experimentally tested. The objective of this study was to investigate the patterns of male mate

choice and differential ejaculate expenditure in relation to MHC similarity during simultaneous and sequential exposure to females, in the sexually promiscuous red junglefowl, *Gallus gallus*.

We designed two experiments to test for differential mating response by a male based on his MHC similarity with a female, under high and low mate availability. The first experiment is a simultaneous mate choice experiment whereby the male is simultaneously presented to two females, an MHC-similar and an MHC-dissimilar female (high mate availability). The second experiment is a sequential mate choice experiment designed to test male mating response according to MHC similarity when females are sequentially presented to a male (low availability). We tested the following two predictions:

- (i) males are more likely to mate with the MHC-dissimilar female and
- (ii) males allocate more sperm to the MHC-dissimilar female.

2. MATERIAL AND METHODS

(a) *Study species*

The mating system of natural populations of red junglefowl including feral populations of its domestic subspecies, *Gallus gallus domesticus*, is characterized by the high opportunity for sexual selection that occurs both before and after copulation (Pizzari *et al.* 2002). Importantly, male fowl bias mating and ejaculate expenditure according to the reproductive quality of a female. First, males preferentially mate with and invest sperm in sexually novel females that they have not inseminated recently (Pizzari *et al.* 2003). Second, females display a sexual ornament, the comb, whose expression covaries, phenotypically and genetically, with different measures of female reproductive investment (Pizzari *et al.* 2003; Cornwallis & Birkhead 2007; Wright *et al.* 2007), and males preferentially mate with—and invest sperm in—females with larger combs (Pizzari *et al.* 2003; Cornwallis & Birkhead 2006). Finally, males show a tendency to avoid genetically related females when unrelated females are simultaneously available (Løvlie & Pizzari, submitted), but invest sperm in these females when unrelated females are not available (Pizzari *et al.* 2004).

(b) *Study populations and MHC typing*

The study was carried out over 3 years: 2005–2007. In 2005 and 2006, the study was conducted on a population of red junglefowl, *G. gallus*, housed at the Swedish University of Agricultural Sciences in Skara (Sweden). This Swedish population was founded from a zoo population originating from Thailand (Schütz *et al.* 2001). The birds were pedigree hatched, using methods identical to those of Pizzari *et al.* (2004), and pedigree data from two generations were available. In 2006, a random-bred stock of the Swedish population was established at the John Krebs Field Station of the University of Oxford, and in 2007, the study was continued on the UK population. No pedigree data were available for this population.

All birds from both the Swedish and Oxford populations were typed for MHC class I and II alleles. The MHC structure of the fowl is well known and simple compared with mammals (Kaufman *et al.* 1999; Jacob *et al.* 2000; Wallny *et al.* 2006; Shaw *et al.* 2007). The MHC *B* haplotype

appears to be inherited as a single unit and contains two MHC class I loci (BF1 and BF2) and two MHC class II loci (BLB1 and BLB2), all encoding antigen-binding molecules (Kaufman *et al.* 1999). The loci BF2 and BLB2 have been shown to be expressed far more than the BF1 and BLB1 loci, respectively, and there is evidence that these dominantly expressed loci determine the immune response to certain infectious pathogens (Kaufman *et al.* 1999; Wallny *et al.* 2006). Thus, BF1 and BLB1 have been termed the minor MHC class I and II loci, respectively, and BF2 and BLB2 have been termed the major MHC class I and II loci, respectively (Kaufman *et al.* 1999; Jacob *et al.* 2000; Wallny *et al.* 2006; Shaw *et al.* 2007).

Several alternative methods have been developed to screen individual genetic variation directly in the domestic chicken, including single-strand conformation polymorphism (Goto *et al.* 2002), automated sequencing (Livant & Ewald 2005) and by using adjacent microsatellites variation (Fulton *et al.* 2006). All these methods have different advantages, but we used the alternative method of reference strand conformation analysis (RSCA). As an automated method, RSCA is suitable for screening large populations reliably, with excellent between-run repeatability even for large fragments (less than 500 bp), and can separate alleles that differ by a single nucleotide (Ramon *et al.* 1998; Kennedy *et al.* 2002).

Four class I and four class II fluorescently labelled reference strands were used for RSCA genotyping, all originating from cloned domestic fowl alleles (further details including GenBank accession numbers can be found in Worley *et al.* 2008). Class I fragments were initially amplified using the primers C71 (5'-CGAGCTCCATACCCCTG CCGTAC-3') and C75 (5'-CTCCTGCCCAGCTCAGC CTTC-3') developed from domestic chicken lines (Shaw *et al.* 2007). Amplicons comprise 767 bp fragments encompassing exons 2 and 3 of both minor and major loci. A 277 bp exon 2 fragment of class II major and minor loci was amplified using the primers OL284BL (5'-GTGCCCGCA GCGTTCTTC-3') and RV280BL (5'-TCCTCTGCACCG TGAAGG-3') (Goto *et al.* 2002). All amplification conditions have been described in Worley *et al.* (2008).

A nested PCR protocol was then devised to allow us to resolve each separate locus. The primers C477 (5'-GTT ACGCCCCGCTTCCCGGTCAACTAC-3') and C69 (5'-GCGGTGCCACTGAGTGCCACCAGGG-3') were used to amplify a fragment of approximately 3.6 kB containing only the class I minor locus (Shaw *et al.* 2007). A second PCR was then performed on the class I minor amplicons using the non specific BF primers C71 and C75. Similarly, the class II minor locus was amplified by the primers C275 (5'-GGTTCCAGGTGCAAGGCGATGGTCTCTGTG CATACCT-3') and C243 (5'-CCATGCCTTGCAGAAAT CGCAGTG-3'), which amplify a 3.4 kB fragment spanning between the adjacent B-loc and Tapasin genes (Jacob *et al.* 2000). Again, a second PCR was performed on these class II minor amplicons using the non specific primers OL284BL and RV280BL as above. All amplification conditions have been described in Worley *et al.* (2008). Therefore variation at all four MHC B loci could be identified independently, which enables the reconstruction of the overall MHC haplotype without the assumption of non-recombination. All jungle fowl alleles identified by RSCA were confirmed and characterized at the nucleotide level by the cloning and sequencing of a subset of individuals (Worley *et al.* 2008).

The Swedish population of 84 individuals contained 9 alleles across the two class I loci (six major and three minor) and 10 alleles across the two class II loci (five major and five minor). The four MHC B loci associated into distinct MHC haplotypes, with no evidence of recombination between loci. Sequence polymorphism was similar to that of domestic fowl at 0.068 for class I and 0.108 for class II loci (Worley *et al.* 2008; table 3) and population expected heterozygosity was variable across loci (class I: minor = 0.57, major = 0.72; class II: minor = 0.65, major = 0.68). The degree of MHC similarity between two individuals was calculated using the following formula: degree of MHC similarity is equal to $(2x)/n$; where x is the number of alleles shared between the two individuals and n is the total number of alleles present in the two individuals.

(c) General experimental protocol

Each male was kept physically isolated from females for at least 48 h prior to each experimental trial in order to control for sperm depletion and test all males with maximal extragonadal sperm reserves (Etches 1996). In each trial, a male was exposed to females held by trained staff. To control for holder effects on male responses to females, females were presented to the male from behind a partition that enabled the male free access to the females but prevented visual contact with the holder. A male was given 1 min to familiarize himself with the females, before females were placed in a soliciting position to enable mating. Manual holding of the female in a soliciting position triggers male mounting and controls for differential female responses to the male, which may influence male sexual behaviour (e.g. Pilastro *et al.* 2004). During each trial, females were fitted with a harness over the cloaca, which enabled the collection of each ejaculate (Pizzari *et al.* 2003, 2004). The ejaculate volume allocated to the female was collected and recorded to the nearest 5 μ l with a pipette. The number of sperm contained in an ejaculate was calculated using standard techniques (Bakst & Cecil 1997; Pizzari *et al.* 2003). Each female was weighed to the nearest 0.1 g and female comb height and length measured to the nearest 0.01 mm with a digital calliper. Female comb area was measured as $(x \times y)/2$, where x is female comb height and y is comb length. We minimized phenotypic differences in comb size and body mass between the two females exposed to the same male, and also controlled for their potential effects statistically (see below).

All analyses were performed using generalized linear mixed models (GLMMs) with restricted maximum-likelihood (REML) estimation in SAS v. 9.1 (Wolfinger & O'Connell 1993). We selected the minimum adequate model using a sequential addition of variables, whereby the model initially contains no variables and variables are added sequentially (Grafen & Hails 2002). At each step, the variable with the lowest p and highest F values was inserted into the model and only variables with $p < 0.05$ were added into the model (Grafen & Hails 2002). The significance of fixed effects was examined using Wald-type tests (type III for main effects and type I for interactions; Grafen & Hails 2002).

(d) Simultaneous mate choice and sperm allocation

This experiment was carried out on 27 males, 11 in 2006 and 16 in 2007. In total, 28 females were used, 12 in 2006 and 16 in 2007. A male and female were considered MHC-dissimilar if the total number of MHC alleles shared was less than 0.18 and similar if more than 0.57. In 2006, each male did not

share grandparents with either female within each trial ($r < 0.0625$). In 2007, pedigree data were not available and relatedness between individuals was unknown. When pairing females, differences in comb size and mass were minimized. Female comb length, comb height and mass did not significantly differ between MHC-similar and MHC-dissimilar females within each experimental pair (paired *t*-test; female comb length: n pairings = 27, $T = -0.16$, $p = 0.874$; female comb height: n pairings = 27, $T = 0.79$, $p = 0.440$; female mass: n pairings = 27, $T = -0.34$, $p = 0.736$). In addition, to control for differences in female comb size and body mass between the two females of a trial, we standardized the value of each of the two females used within a trial against the average value for that trial (e.g. comb size of female x was calculated as: $(x-y)/((x+y)/2)$, where x is the comb size of female x and y is the comb size of female y).

The two females were first simultaneously exposed to a male for 1 min in an upright position to allow him to familiarize himself with the females. Females were then presented simultaneously to the male in a soliciting position for 20 min or until he copulated with one of the females. In two instances, the male did not copulate with either female within 20 min, and the trial was repeated, at least 48 h later. The female with whom the male copulated first was considered the 'preferred' female of the two. A male was deemed to have copulated with a female if cloaca contact was observed, and sperm allocation was measured by the number of sperm ejaculated by the male. If a male copulated with a female but did not ejaculate any sperm, sperm allocation to this particular copulation was recorded as zero. Immediately after the first presentation, the male was presented once again with the same two females but this time a wired cage was placed over the preferred female, that prevented physical but not visual contact with her, and forced the male to copulate with the female with whom he did not copulate in the first presentation ('least preferred' female, see: Pizzari *et al.* 2003).

To control for copulation order effects on sperm allocation, a second trial was conducted with the same females after a male was sexually rested. In the second trial, males were forced to copulate in the reverse order of the first trial: with the least preferred female first and the preferred female second. Therefore, in the second trial, a male was again given the possibility to copulate with each female, but in this case copulation order was predetermined and reversed compared to his first trial. Once again, the amount of sperm allocated to each female was quantified.

We carried out the analysis in two parts. First, we tested whether males preferentially copulate with an MHC-dissimilar female (i.e. whether they copulated first with the MHC-dissimilar female in the first trial) through a χ^2 -test. Second, we investigated whether the number of sperm in the ejaculate allocated to each female varied according to MHC similarity through a GLMM with residual pseudo-likelihood estimation, a Poisson error distribution and log link function, forward selection of non-significant terms, the number of sperm allocated to a female in a trial as the dependent variable, MHC similarity category (similar or dissimilar) and copulation order (first or second with female) as fixed factors. Standardized female comb size and standardized female mass were entered as covariates, while trial (first allocation or second allocation trial), male identity (nested within trial), female identity and the interaction of male and female identity and year of experiment were entered as random

factors. The fixed effect with the highest p value was sequentially added until only significant terms ($p > 0.05$) remained in the model.

In order to explore more fully the possibility that any MHC similarity effect could be mediated by a single area or a subset of the MHC, and determine the extent to which the two MHC classes contribute to such effects, we replaced the index of MHC similarity in the model with indices of similarity based on the subsets of the MHC loci, i.e. (i) the number of alleles shared by a male and a female in MHC class I and (ii) class II separately, (iii) the combined number of MHC major alleles shared, (iv) the number of MHC major alleles shared in class I and (v) class II separately. Finally, MHC genes are thought to be inherited as a unit (i.e. a haplotype) as recombination between the class I and II genes was not observed in over 6000 domestic fowl progeny (Skjoldt *et al.* 1985). We also observed no evidence of recombination in the jungle fowl populations, as independently amplified MHC loci associated into distinct haplotypes. Consequently, genes tightly linked to the MHC genes, such as olfactory receptor (OR) genes, may mediate MHC haplotype recognition rather than individual MHC allele recognition (Penn & Potts 1999). We, therefore, also tested the effect of MHC similarity measured as the proportion of total number of haplotypes shared. Because two birds can only share both, one or zero haplotypes, this index had only three values: 1.0; 0.5; and 0.0, respectively. It is important to note that during forward selection of explanatory variables in the models, different MHC similarity measures were considered as alternative explanatory variables owing to their collinearity. Therefore, different MHC similarity measures were never entered simultaneously in the same model. Akaike information criterion (AIC, Akaike 1973, 1987; Bozdogan 1987, 2000; SAS 2006) values were used to compare the adequacy of alternative models in which different MHC similarity measures were significant explanatory variables. The difference in AIC values between these models was considered significant when greater than 1 (Akaike 1973, 1987; Bozdogan 1987, 2000; SAS 2006).

(e) *Sequential mate choice and sperm allocation*

This experiment was conducted on 27 males, 14 in 2005, 10 in 2006 and 8 in 2007 (some males were used both in 2005 and 2006). In total 42 females were used, 21 in 2005, 17 in 2006 and 10 in 2008 (six females were used both in 2005 and 2006). In each trial, a male was exposed to a single female and allowed to copulate with her. We measured male propensity to copulate as the time (to the nearest 0.5 min) it took a male to copulate with a female immediately after she was presented to him. Males sometimes mount females without allocating any sperm to the female (Pizzari *et al.* 2003; Lovlie *et al.* 2005). To control for this possibility, males were allowed to copulate up to two times with a female during a trial of 20 min.

First, we investigated male propensity to copulate in relation to MHC similarity, by analysing the time elapsed to first copulation in relation to MHC similarity through a REML GLMM with a lognormal error distribution and identity link function, time elapsed to first copulation as the dependent variable, the MHC similarity (see below), female mass and female comb area as covariates and male and female identity and year of experiment as random factors. Second, we investigated male differential ejaculate expenditure according to MHC similarity, by analysing the number of

Table 1. Sources of variance in the number of sperm allocated by a male to a female in the simultaneous mate choice experiment ($n_{\text{males}}=27$), degrees of freedom (d.f.), F ratio (F) and significance level (p). (Values in bold represent p -values <0.05 .)

model	d.f.	F	p -value
MHC category	1, 26	3.01	0.094
copulation order	1, 26	34.69	<0.001
standardized female comb size	1, 26	0.66	0.422
standardized female mass	1, 26	0.21	0.653
trial	1, 52	2.37	0.13

sperm in the ejaculate allocated to each female according to MHC similarity through a REML GLMM with a Gaussian error distribution and identity link function, the number of sperm allocated to a female in a trial as the dependent variable, the number of copulations during trial (one or two copulations) as fixed factors; male and female identity and year of experiment as random factors; female mass, female comb area and MHC similarity, measured as the number of MHC alleles shared by a male and a female across both major and minor loci of each class combined, as covariates. Once again, we further explored the mechanisms underlying the potential effects of MHC similarity by replacing MHC similarity in the model with (i) the number of alleles shared by a male and a female in MHC class I and (ii) class II separately, (iii) the combined number of MHC major alleles shared, (iv) the number of MHC major alleles shared in class I and (v) class II separately and (vi) the proportion of total number of haplotypes shared, as outlined above.

We carried out each analysis (i.e. mating propensity and ejaculate expenditure) using the data collected from both the Swedish population and the UK population. We then repeated each analysis focusing exclusively on the data collected from the Swedish population, for which pedigree data were available, entering pedigree relatedness (a male and a female were either full-sib, $r=0.5$, or unrelated, $r<0.0625$, to each other) as an additional fixed factor, which enabled us to control for the potential effects of relatedness.

3. RESULTS

(a) *Simultaneous mate choice and sperm allocation*

Males were not more likely to initiate a copulation with an MHC-dissimilar female first ($X^2_1=0.054$; $p=0.816$); 13 males copulated with the MHC-similar female first and 14 males copulated with the MHC-dissimilar females first.

After controlling for copulation order, males did not allocate sperm differentially according to their MHC similarity with a female (table 1).

(b) *Sequential mate choice and sperm allocation*

Males did not show a higher propensity to copulate with MHC-dissimilar females (table 2a). This result was confirmed when considering only the data from the Swedish population. After controlling for genetic relatedness between a male and a female, female comb size and body mass, we did not find a significant relationship between male propensity to copulate and MHC similarity (table 2b).

Males allocated significantly more sperm to the female with whom they were most MHC-dissimilar (table 3a;

figure 1). All measures of MHC similarity were significant when the effect of each was entered separately into the model. However, based on the comparison of AIC values between the models, the measure of MHC similarity that explained the most variance was the total number of MHC haplotypes shared by a male and a female ($F_{1,11}=6.97$; $p=0.023$; figure 1). This result was not significant when analysing the smaller dataset from the Swedish population only (table 3b).

4. DISCUSSION

This study investigated patterns of overt and cryptic male mating preferences in relation to the MHC similarity of a female, during both sequential and simultaneous mate choice. We found no evidence of overt male mating preference. However, while the males were just as likely to mate with an MHC-dissimilar as with an MHC-similar female, they showed some cryptic preference by allocating more sperm to MHC-dissimilar females during sequential mate choice.

Previous work has demonstrated male mating preferences in the fowl. First, male feral fowl, *G. g. domesticus*, are more likely to mate with a female with a larger comb (Cornwallis & Birkhead 2007), a trait phenotypically (Pizzari *et al.* 2003; Cornwallis & Birkhead 2007) and genetically (Wright *et al.* 2007) correlated with female reproductive investment. Second, male red junglefowl display kin recognition and avoid full-sib sisters when unrelated females are available (Løvlie & Pizzari submitted), but eagerly mate with full-sib sisters when unrelated females are not available (Pizzari *et al.* 2004; Løvlie & Pizzari submitted). However, the full extent of male preference based on female comb size and genetic relatedness is revealed only when considering 'cryptic' male preferences in terms of differential ejaculate expenditure (Pizzari *et al.* 2003, 2004; Cornwallis & Birkhead 2007). Consistent with previous work, the results of the present study suggest that male fowl might show a preference for MHC-dissimilar partners but only in terms of ejaculate expenditure and only when a male encounters individual females sequentially.

While the proximate mechanisms underlying recognition of MHC similarity are unknown in birds, studies in other vertebrates suggest that it may be mediated by olfactory cues (e.g. Fan *et al.* 1995; Wedekind *et al.* 1995; Wedekind & Furi 1997; Yamazaki *et al.* 1999; Reusch *et al.* 2001; Carroll *et al.* 2002; Aeschlimann *et al.* 2003; Olsson *et al.* 2003; Hurst *et al.* 2005; Milinski *et al.* 2005). For example, both mice (Yamazaki *et al.* 1999; Carroll *et al.* 2002) and rats (Brown *et al.* 1987) are able to discriminate between the urinary odour of an MHC-dissimilar and an MHC-similar individual, although in mice there seems to be a learnt component to MHC recognition (Beauchamp & Yamazaki 2003; Hurst *et al.* 2005). As in mice, MHC recognition in humans appears to be mediated by odour cues because both males and females prefer the odour of MHC-dissimilar individuals (Wedekind *et al.* 1995; Wedekind & Furi 1997) and polymorphic OR genes are located in the MHC (Fan *et al.* 1995). Another example of odour-mediated MHC recognition was provided by a study of sand lizards, *Lacerta agilis*, which experimentally demonstrated that the females preferred to associate with odour samples

Table 2. Sources of variation in mate preference in the sequential experiment for (a) the overall dataset ($n_{\text{males}} = 27$) and (b) the Swedish population only ($n_{\text{males}} = 19$). (AIC value was used to select the best model, with smaller AIC indicating a better-fit model. The difference between the two AIC values was considered insignificant when equal to or less than 1.)

model	AIC	d.f.	<i>F</i>	<i>p</i> -value
(a) the overall dataset ($n_{\text{males}} = 27$)				
MHC alleles shared ^a	307.2	1, 12	0.29	0.599
MHC haplotypes shared	307.13	1, 12	0.33	0.574
female comb size	317.6	1, 13	1.44	0.251
female mass	304.96	1, 13	0.48	0.502
(b) the Swedish population only ($n_{\text{males}} = 19$)				
relatedness	215.75	1, 4	5.81	0.074
MHC alleles shared ^a	214.27	1, 3	0.04	0.847
MHC haplotypes shared	206.28	1, 3	0.03	0.875
female comb size	224.6	1, 4	2.27	0.206
female mass	211.57	1, 3	1.04	0.383

^aAlternative MHC similarity measures, MHC class I alleles shared, MHC class II alleles shared, MHC major alleles shared, MHC major I alleles shared and MHC major II alleles shared did not significantly predict mate preference in the sequential experimental.

Table 3. Sources of variation in the number of sperm ejaculated by a male in the sequential experiment. ((a) The overall dataset ($n_{\text{males}} = 27$) and (b) Swedish population only ($n_{\text{males}} = 19$). AIC values were used to select the best model, with smaller AIC indicating a better-fit model. The difference between the two AIC values was considered insignificant when equal to or less than 1. Values in bold represent *p*-values < 0.05.)

model	AIC	d.f.	<i>F</i>	<i>p</i> -value
(a) the overall dataset ($n_{\text{males}} = 27$)				
total MHC alleles shared ^a	2983.92	1, 11	5.82	0.035
MHC haplotypes shared ^b	2982.87	1, 11	6.97	0.023
comb size	2957.91	1, 11	0.77	0.4
female mass	2942.52	1, 11	2.77	0.124
(b) Swedish population only ($n_{\text{males}} = 19$)				
relatedness	2075	1, 4	1.19	0.336
total MHC alleles shared ^c	2079.68	1, 4	1.24	0.328
MHC haplotypes shared	2078.68	1, 4	2.36	0.199
comb size	2093.01	1, 4	0.04	0.843
female mass	2077.82	1, 4	1.21	0.333

^aWhen comparing AIC values between the models no other alternative MHC similarity measures, MHC class I alleles shared, MHC class II alleles shared, MHC major alleles shared, MHC major I alleles shared and MHC major II alleles shared were a significantly better fit in explaining variation in number of sperm ejaculated when using the overall dataset.

^bMHC haplotypes shared explained significantly more of the variance in the number of sperm ejaculated than all other MHC similarity measures, MHC class I alleles shared, MHC class II alleles shared, MHC major alleles shared, MHC major I alleles shared and MHC major II alleles shared, when comparing AIC values and using the overall dataset.

^cAlternative MHC similarity measures, MHC class I alleles shared, MHC class II alleles shared, MHC major alleles shared, MHC major I alleles shared and MHC major II alleles shared did not significantly predict variation in the number of sperm when restricting the analysis to the Swedish population.

obtained from MHC-dissimilar males at the class I loci (Olsson *et al.* 2003). The fowl MHC exhibits a low level of recombination, giving rise to distinct haplotypes that are relatively conserved in evolution (Skjdt *et al.* 1985; Kaufman *et al.* 1999). Therefore, genes tightly linked to the MHC *B* region, such as olfactory genes, may mediate MHC genotype recognition (Penn & Potts 1999). It has recently been found that there are 218 non-identical fowl genes that are predicted to be orthologous to one of two OR gene clusters in humans, which are positioned next to the MHC class I gene clusters (International Chicken Genome Sequencing Consortium 2004). Furthermore, there are at least 554 OR genes (Aloni *et al.* 2006), challenging the traditional view that birds have a poor sense of smell (Alcock 1989). The high number of OR genes may indicate that olfactory cues are important in the fowl. The fact that the measure of MHC similarity, which explained most of the variance in terms of ejaculate expenditure, is the number of MHC haplotypes shared by

a male and a female supports the hypothesis that MHC recognition may be mediated by alleles closely linked to the MHC *B* complex.

Theory predicts individuals to prefer reproductive partners of different MHC genotypes (Penn & Potts 1999; Milinski 2006). In addition to MHC heterozygosity, a preference for MHC-dissimilar partners may also be explained by inbreeding avoidance (Brown & Eklund 1994). We used the dataset of the Swedish population, for which MHC and genetic relatedness information was available, to attempt to disentangle the effects of MHC similarity *per se* from the effect of inbreeding avoidance. The effect of MHC similarity detected in the sequential mate choice experiment disappeared when we restricted the analysis to the Swedish population. However, it is debatable whether this result can be explained by the effects of genetic relatedness for two reasons. First, we did not detect a relatedness effect in this dataset. Second, previous work on this population indicated that males show only a weak

Figure 1. Differential male ejaculate expenditure based on MHC similarity. The number of sperm allocated by a male to a female during the sequential mate choice experiment, in relation to the proportion of MHC haplotypes shared.

tendency to inseminate more (rather than fewer) sperm when exposed to full-sib sisters. Because related individuals are more likely to have similar MHC, this response would counteract rather than explain the male cryptic preference for MHC-dissimilar females detected by the analysis of the whole dataset. Instead, it is possible that restricting the analysis to a subset of the dataset may have reduced its power, preventing its ability to detect relatedness and MHC-similarity effects.

Theory would also predict males to become more selective as the availability of females increases (Parker 1983; Sullivan 1994; Deutsch & Reynolds 1995; Johnstone *et al.* 1996; Kokko & Monaghan 2001; Kokko & Ots 2006), and thus we would expect this preference to be more pronounced under simultaneous rather than sequential mate choice. Contrary to this expectation, we found that males biased ejaculate expenditure when they were exposed to only one (rather than two) female at a time. A potentially confounding factor may be the social status of a male, which has been shown to influence strategies of differential ejaculate expenditure in this species (Pizzari *et al.* 2003; Cornwallis & Birkhead 2006). Dominant males are often exposed to lower risk and intensity of sperm competition, and will bias ejaculate expenditure in favour of the more ornamented of two simultaneously presented females, while this bias disappears when females are presented sequentially, as predicted by the theory (Cornwallis & Birkhead 2006). Subdominant males, on the other hand, typically experience limited access to females and do not bias ejaculate expenditure under simultaneous nor sequential female exposure (Cornwallis & Birkhead 2006). However, our study tested individual males singly, in isolation from competitors when any influence of status on ejaculate expenditure tends to disappear (e.g. Pizzari *et al.* 2003), and the present results are therefore unlikely to be influenced by social status. Clearly, the adaptive significance of the patterns of cryptic male preference revealed by this study warrants further research.

Regardless of the underlying functional mechanisms, the possibility that males bias ejaculate expenditure in relation to their genetic compatibility with a female has some important implications for the study of female preferences. Three lines of arguments have been interpreted as evidence that females prefer MHC-dissimilar males. First, mate choice trials have shown some evidence for higher female propensity to mate with MHC-dissimilar partners in a range of species (e.g. humans: Wedekind

et al. 1995; Ober *et al.* 1997; Wedekind & Furi 1997; rodents: Arcaro & Eklund 1998; Jordan & Bruford 1998; Penn & Potts 1999; fishes: Landry *et al.* 2001; Reusch *et al.* 2001; Aeschlimann *et al.* 2003; Wegner *et al.* 2003; Milinski *et al.* 2005; Jager *et al.* 2007; sand lizards, *L. agilis*: Olsson *et al.* 2003). Second, in some natural populations, females are more likely to form social bonds with MHC-dissimilar partners, and/or seek extra-pair copulations with males that are more MHC-dissimilar than their social partners (e.g. passerine birds: Freeman-Gallant *et al.* 2002; Richardson *et al.* 2005; Bonneaud *et al.* 2006). Third, paternity is sometimes biased in favour of MHC-dissimilar parents (e.g. Atlantic salmon, *Salmo salar*: Consuegra & de Leaniz 2008; passerines: Freeman-Gallant *et al.* 2003; Richardson *et al.* 2005; grey mouse lemur, *Microcebus murinus*: Schwensow *et al.* 2008). However, the relative number of sperm inseminated by competing males into a female is a well-known predictor of fertilization success under sperm competition in the fowl (Martin *et al.* 1974), as in several other species (e.g. Gage & Morrow 2003). Biases in male ejaculate expenditure are, therefore, likely to influence fertilization success and variation in paternity might be influenced by cryptic male preference, thus calling into question the interpretation of the second and third approaches outlined above.

More generally, the patterns of variation in paternity within broods are often used to study cryptic female choice, particularly in relation to genetic compatibility. While the functional significance of female remating remains debated (Pizzari & Snook 2004; Arnqvist & Kirkpatrick 2005; Akcay & Roughgarden 2007; Griffith 2007), the results of our study indicate that males too can cryptically bias fertilization success in relation to the genetic compatibility of a female. Future studies would need to experimentally disentangle the effect of male- and female-driven bias in paternity to better understand mate preferences and their functional implications.

The work was funded by a Biotechnology and Biological Sciences Research Council studentship to M.A.F.G., and a Natural Environment Research Council research grant to T.P. and D.S.R. (NE/D002788/1). We are grateful to Dom Cram and Dom Wright for their field assistance.

REFERENCES

- Aeschlimann, P. B., Häberli, 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. (doi:10.1007/s00265-003-0611-6)
- Akaike, H. 1973 Information theory and an extension of the maximum likelihood principle. In *Proceedings of the second international symposium on information theory* (eds B. N. Petov & F. Caski), pp. 267–281. Budapest: Akademiai Kiado.
- Akaike, H. 1987 Factor analysis and AIC. *Psychometrika* **52**, 317–332. (doi:10.1007/BF02294359)
- Akcay, E. & Roughgarden, J. 2007 Extra-pair parentage: a new theory based on transactions in a cooperative game. *Evol. Ecol. Res.* **9**, 1223–1243.
- Alcock, J. 1989 *Animal behavior: an evolutionary approach*. Sunderland, MA: Sinauer Associates.
- Aloni, R., Olender, T. & Lancet, D. 2006 Ancient genomic architecture for mammalian olfactory receptor clusters. *Genome Biol.* **7**, R88. (doi:10.1186/gb-2006-7-10-r88)

- Amundsen, T. & Forsgren, E. 2001 Male mate choice selects for female coloration in a fish. *Proc. Natl Acad. Sci. USA* **98**, 13 155–13 160. (doi:10.1073/pnas.211439298)
- Arcaro, K. F. & Eklund, A. 1998 A review of MHC-based mate preferences and fostering experiments in two congenic strains of mice. *Genetica* **104**, 241–244. (doi:10.1023/A:1026465421201)
- Arnqvist, G. & Kirkpatrick, M. 2005 The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. *Am. Nat.* **165**, S26–S37. (doi:10.1086/429350)
- Bakst, M. R. & Cecil, H. C. 1997 Techniques for semen evaluation, semen storage, and fertility determination 4. Sperm motility and metabolism VII. In *In vitro sperm-egg interaction assay utilising inner perivitelline layer from laid chicken eggs*. Savoy, IL: Poultry Science Association.
- Ball, M. A. & Parker, G. A. 2003 Sperm competition games: sperm selection by females. *J. Theor. Biol.* **224**, 27–42. (doi:10.1016/S0022-5193(03)00118-8)
- Beauchamp, G. K., Yamazaki, K., Bard, J. & Boyse, E. A. 1988 Prewaning experience in the control of mating preferences by genes in the major histocompatibility complex of the mouse. *Behav. Genet.* **18**, 537–547. (doi:10.1007/BF01065520)
- Beauchamp, G. K. & Yamazaki, K. 2003 Chemical signalling in mice. *Biochem. Soc. Trans.* **31**, 147–151.
- Bonduriansky, R. 2001 The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**, 305–339. (doi:10.1017/S1464793101005693)
- Bonneaud, C., Chastel, O., Federici, P., Westerdaal, H. & Sorci, G. 2006 Complex Mhc-based mate choice in a wild passerine. *Proc. R. Soc. B* **273**, 1111–1116. (doi:10.1098/rspb.2005.3325)
- Bozdogan, H. 1987 Model selection and Akaike's information criterion (AIC): the general theory and its analytical extensions. *Psychometrika* **52**, 345–370. (doi:10.1007/BF02294361)
- Bozdogan, H. 2000 Akaike's information criterion and recent developments in information complexity. *J. Math. Psychol.* **44**, 62–91. (doi:10.1006/jmps.1999.1277)
- Bretman, A., Wedell, N. & Tregenza, T. 2004 Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. B* **271**, 159–164. (doi:10.1098/rspb.2003.2563)
- Brown, J. L. 1983 Some paradoxical goals of cells and organisms: the role of the MHC. In *Ethical questions in brain and behavior* (ed. D. W. Pfaff), pp. 111–124. New York, NY: Springer.
- Brown, R. E., Singh, P. B. & Roser, B. 1987 The Major Histocompatibility Complex and the chemosensory recognition of individuality in rats. *Physiol. Behav.* **40**, 65–73. (doi:10.1016/0031-9384(87)90186-7)
- Brown, J. L. & Eklund, A. 1994 Kin recognition and the major histocompatibility complex—an integrative review. *Am. Nat.* **143**, 435–461. (doi:10.1086/285612)
- Burley, N. 1977 Parental investment, mate choice, and mate quality. *Proc. Natl Acad. Sci. USA* **74**, 3476–3479. (doi:10.1073/pnas.74.8.3476)
- Byrne, P. G. & Rice, W. R. 2006 Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. *Proc. R. Soc. B* **273**, 917–922. (doi:10.1098/rspb.2005.3372)
- Carroll, L. S., Penn, D. J. & Potts, W. K. 2002 Discrimination of MHC-derived odors by untrained mice is consistent with divergence in peptide-binding region residues. *Proc. Natl Acad. Sci. USA* **99**, 2187–2192. (doi:10.1073/pnas.042244899)
- Chenoweth, S. F., Doughty, P. & Kokko, H. 2006 Can non-directional male mating preferences facilitate honest female ornamentation? *Ecol. Lett.* **9**, 179–184. (doi:10.1111/j.1461-0248.2005.00867.x)
- Chenoweth, S. F., Petfield, D., Doughty, P. & Blows, M. W. 2007 Male choice generates stabilizing sexual selection on a female fecundity correlate. *J. Evol. Biol.* **20**, 1745–1750. (doi:10.1111/j.1420-9101.2007.01390.x)
- Colegrave, N., Kotiaho, J. S. & Tomkins, J. L. 2002 Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol. Ecol. Res.* **4**, 911–917.
- Consuegra, S. & de Leaniz, C. G. 2008 MHC-mediated mate choice increases parasite resistance in salmon. *Proc. R. Soc. B* **275**, 1397–1403. (doi:10.1098/rspb.2008.0066)
- Cornwallis, C. K. & Birkhead, T. R. 2006 Social status and availability of females determine patterns of sperm allocation in the fowl. *Evolution* **60**, 1486–1493. (doi:10.1111/j.0014-3820.2006.tb01227.x)
- Cornwallis, C. K. & Birkhead, T. R. 2007 Experimental evidence that female ornamentation increases the acquisition of sperm and signals fecundity. *Proc. R. Soc. B* **274**, 583–590. (doi:10.1098/rspb.2006.3757)
- Deutsch, J. C. & Reynolds, J. D. 1995 Design and sexual selection: the evolution of sex differences in mate choice. In *Perspectives in ethology* (ed. N. S. Thompson) *Behavioral design*, vol. 11, pp. 297–323. New York, NY: Plenum Press.
- Eklund, A., Egid, K. & Brown, J. L. 1991 The major histocompatibility complex and mating preferences of male-mice. *Anim. Behav.* **42**, 693–694. (doi:10.1016/S0003-3472(05)80254-4)
- Etches, R. J. 1996 *Reproduction in poultry*. Oxford, UK: CAB International.
- Fan, W. F., Liu, Y. C., Parimoo, S. & Weissman, S. M. 1995 Olfactory receptor-like genes are located in the human major histocompatibility complex. *Genomics* **27**, 119–123. (doi:10.1006/geno.1995.1013)
- Freeman-Gallant, C. R., Johnson, E. M., Saponara, F. & Stanger, M. 2002 Variation at the major histocompatibility complex in Savannah sparrows. *Mol. Ecol.* **11**, 1125–1130. (doi:10.1046/j.1365-294X.2002.01508.x)
- 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. (doi:10.1046/j.1365-294X.2003.01968.x)
- Fulton, J. E., Juul-Madsen, H. R., Ashwell, C. M., McCarron, A. M., Arthur, J. A., O'Sullivan, N. P. & Taylor Jr, R. L. 2006 Molecular genotype identification of the *Gallus gallus* major histocompatibility complex. *Immunogenetics* **58**, 407–421. (doi:10.1007/s00251-006-0119-0)
- Gage, M. J. G. & Morrow, E. H. 2003 Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. *Curr. Biol.* **13**, 754–757. (doi:10.1016/S0960-9822(03)00282-3)
- Glick, B. 2000 Immunophysiology. In *Sturkie's avian physiology* (ed. G. C. Whittow), pp. 657–685. San Diego, CA: Academic Press.
- Goto, R., Afanassieff, M., Ha, J., Iglesias, G. M., Ewald, S. J., Briles, W. E. & Miller, M. M. 2002 Single-strand conformation polymorphism (SSCP) assays for major histocompatibility complex B genotyping in chickens. *Poult. Sci.* **81**, 1832–1841.
- Grafen, A. & Hails, R. 2002 *Modern statistics for the life sciences*. Oxford, UK: Oxford University Press.
- Griffith, S. C. 2007 The evolution of infidelity in socially monogamous passerines: Neglected components of direct and indirect selection. *Am. Nat.* **169**, 274–281. (doi:10.1086/510601)
- Hoffman, J. I., Forcada, J., Trathan, P. N. & Amos, W. 2007 Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* **445**, 912–914. (doi:10.1038/nature05558)

- Hurst, J. L., Thom, M. D., Nevison, C. M., Humphries, R. E. & Beynon, R. J. 2005 MHC odours are not required or sufficient for recognition of individual scent owners. *Proc. R. Soc. B* **272**, 715–724. (doi:10.1098/rspb.2004.3004)
- International Chicken Genome Sequencing Consortium 2004 *Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution*. *Nature* **432**, 695–716. (doi:10.1038/nature03154)
- Jacob, J. P., Milne, S., Beck, S. & Kaufman, J. 2000 The major and a minor class II beta-chain (B-LB) gene flank the *Tapasin* gene in the B-F/B-L region of the chicken major histocompatibility complex. *Immunogenetics* **51**, 138–147. (doi:10.1007/s002510050022)
- Jager, I., Eizaguirre, C., Griffiths, S. W., Kalbe, M., Krobach, C. K., Reusch, T. B. H., Schaschl, H. & Milinski, M. 2007 Individual MHC class I and MHC class IIB diversities are associated with male and female reproductive traits in the three-spined stickleback. *J. Evol. Biol.* **20**, 2005–2015. (doi:10.1111/j.1420-9101.2007.01366.x)
- Janeway, C., Travers, P., Walshport, M. & Capra, J. 1999 *Immunobiology: the immune system in health and disease*. London, UK: Elsevier Science Ltd.
- Jehle, R., Szatecsny, M., Wolf, J. B. W., Whitlock, A., Hodl, W. & Burke, T. 2007 Genetic dissimilarity predicts paternity in the smooth newt (*Lissotriton vulgaris*). *Biol. Lett.* **3**, 526–528. (doi:10.1098/rsbl.2007.0311)
- Jennions, M. D., Hunt, J., Graham, R. & Brooks, R. 2004 No evidence for inbreeding avoidance through postcopulatory mechanisms in the black field cricket, *Teleogryllus commodus*. *Evolution* **58**, 2472–2477. (doi:10.1111/j.0014-3820.2004.tb00876.x)
- Johnstone, R. A., Reynolds, J. D. & Deutsch, J. C. 1996 Mutual mate choice and sex differences in choosiness. *Evolution* **50**, 1382–1391. (doi:10.2307/2410876)
- Jones, I. L. & Hunter, F. M. 1993 Mutual sexual selection in a monogamous seabird. *Nature* **362**, 238–239. (doi:10.1038/362238a0)
- Jordan, W. C. & Bruford, M. W. 1998 New perspectives on mate choice and the MHC. *Heredity* **81**, 127–133. (doi:10.1038/sj.hdy.6884281)
- Kaufman, J., Milne, S., Gobel, T. W. F., Walker, B. A., Jacob, J. P., Auffray, C., Zoorob, R. & Beck, S. 1999 The chicken B locus is a minimal essential major histocompatibility complex. *Nature* **401**, 923–925. (doi:10.1038/44856)
- Kempnaers, B. 2007 Mate choice and genetic quality: a review of the heterozygosity theory. *Adv. Study Behav.* **37**, 189–278. (doi:10.1016/S0065-3454(07)37005-8)
- Kennedy, L. J., Ryvar, R., Gaskell, R. M., Addie, D. D., Willoughby, K., Carter, S. D., Thomson, W., Ollier, W. E. R. & Radford, A. D. 2002 Sequence analysis of MHC DRB alleles in domestic cats from the United Kingdom. *Immunogenetics* **54**, 348–352. (doi:10.1007/s00251-002-0465-5)
- Kokko, H. & Monaghan, P. 2001 Predicting the direction of sexual selection. *Ecol. Lett.* **4**, 159–165. (doi:10.1046/j.1461-0248.2001.00212.x)
- Kokko, H. & Ots, I. 2006 When not to avoid inbreeding. *Evolution* **60**, 467–475. (doi:10.1554/05-613.1)
- Kotiaho, J. S., Lebas, N. R., Puurtinen, M. & Tomkins, J. L. 2008 On female choice, heterozygosity and the lek paradox. *Anim. Behav.* **75**, E1–E3. (doi:10.1016/j.anbehav.2007.08.011)
- 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. (doi:10.1098/rspb.2001.1659)
- Livant, E. J. & Ewald, S. J. 2005 High-resolution typing for chicken BF2 (MHC class I) alleles by automated sequencing. *Anim. Genet.* **36**, 432–434. (doi:10.1111/j.1365-2052.2005.01331.x)
- Lovlie, H., Cornwallis, C. K. & Pizzari, T. 2005 Male mounting alone reduces female promiscuity in the fowl. *Curr. Biol.* **15**, 1222–1227. (doi:10.1016/j.cub.2005.05.060)
- Martin, P. A., Reimers, T. J., Lodge, J. R. & Dziuk, P. J. 1974 The effect of ratios and numbers of spermatozoa mixed from two males on proportions of offspring. *J. Reprod. Fert.* **39**, 251–258. (doi:10.1530/jrf.0.0390251)
- Milinski, M. 2006 The major histocompatibility complex, sexual selection, and mate choice. *Annu. Rev. Ecol. Evol. Syst.* **37**, 159–186. (doi:10.1146/annurev.ecolsys.37.091305.110242)
- Milinski, M., Griffiths, S., Wegner, K. M., Reusch, T. B. H., Haas-Assenbaum, A. & Boehm, T. 2005 Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proc. Natl Acad. Sci. USA* **102**, 4414–4418. (doi:10.1073/pnas.0408264102)
- Neff, B. D. & Pitcher, T. E. 2005 Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* **14**, 19–38. (doi:10.1111/j.1365-294X.2004.02395.x)
- Ober, C., Weitkamp, N., Cox, H., Dytych, D., Kostyu, D. & Elias, S. 1997 HLA and mate choice in humans. *Am. J. Hum. Genet.* **61**, 497–504. (doi:10.1086/515511)
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstrom, H. 1996 Sperm selection by females. *Nature* **383**, 585. (doi:10.1038/383585a0)
- Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B. & Wittsell, H. 2003 Major histocompatibility complex and mate choice in sand lizards. *Proc. R. Soc. B* **270**, S254–S256. (doi:10.1098/rsbl.2003.0079)
- Owens, I. P. F. & Thompson, D. B. A. 1994 Sex-differences, sex-ratios and sex-roles. *Proc. R. Soc. B* **258**, 93–99. (doi:10.1098/rspb.1994.0148)
- Parker, G. A. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (eds M. S. Blum & N. A. Blum), pp. 123–166. New York, NY: Academic Press.
- Parker, G. A. 1983 Male quality and mating decisions. In *Mate choice* (ed. P. Bateson), pp. 141–166. New York, NY: Cambridge University Press.
- Parker, G. A. 2006 Sexual conflict over mating and fertilization: an overview. *Phil. Trans. R. Soc. B* **361**, 235–259. (doi:10.1098/rstb.2005.1785)
- Penn, D. J. & Potts, W. K. 1999 The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat.* **153**, 145–164. (doi:10.1086/303166)
- Pilastro, A., Simonato, M., Bisazza, A. & Evans, J. P. 2004 Cryptic female preferences for colorful males in guppies. *Evolution* **58**, 665–669. (doi:10.1554/03-491)
- Pizzari, T. & Bonduriansky, R. 2009 Sexual behaviour: conflict, cooperation and co-evolution. In *Social behaviour: genes, ecology and evolution* (eds T. Szekely, A. J. Moore & J. Komdeur). Cambridge, UK: Cambridge University Press.
- Pizzari, T., Froman, D. P. & Birkhead, T. R. 2002 Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. *Heredity* **88**, 112–116. (doi:10.1038/sj.hdy.6800014)
- Pizzari, T., Cornwallis, C. K., Lovlie, H., Jakobsson, S. & Birkhead, T. R. 2003 Sophisticated sperm allocation in male fowl. *Nature* **426**, 70–74. (doi:10.1038/nature02004)
- Pizzari, T., Lovlie, H. & Cornwallis, C. K. 2004 Sex-specific, counteracting responses to inbreeding in a bird. *Proc. R. Soc. B* **271**, 2115–2121. (doi:10.1098/rspb.2004.2843)

- Pizzari, T. & Snook, R. R. 2004 Sexual conflict and sexual selection: Measuring antagonistic coevolution. *Evolution* **58**, 1389–1393. (doi:10.1554/04-085)
- Potts, W. K. & Wakeland, E. K. 1993 Evolution of Mhc genetic diversity—a tale of incest, pestilence and sexual preference. *Trends Genet.* **9**, 408–412. (doi:10.1016/0168-9525(93)90103-O)
- Ramon, D. S., Argüello, J. R., Cox, S. T., McWhinnie, A., Little, A.-M., Marsh, S. G. E. & Madrigal, J. A. 1998 Application of RSCA for typing of HLA-DPB1. *Hum. Immunol.* **59**, 734–747. (doi:10.1016/S0198-8859(98)00079-2)
- Reinhold, K., Kurtz, J. & Engqvist, L. 2002 Cryptic male choice: sperm allocation strategies when female quality varies. *J. Evol. Biol.* **15**, 201–209. (doi:10.1046/j.1420-9101.2002.00390.x)
- 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. (doi:10.1038/35104547)
- Richardson, D. S., 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. B* **272**, 759–767. (doi:10.1098/rspb.2004.3028)
- Rubolini, D., Galeotti, P., Ferrari, G., Spairani, M., Bernini, F. & Fasola, M. 2006 Sperm allocation in relation to male traits, female size, and copulation behaviour in a freshwater crayfish species. *Behav. Ecol. Sociobiol.* **60**, 212–219. (doi:10.1007/s00265-005-0158-9)
- SAS 2006 *The GLIMMIX procedure*. Cary, NC: SAS publishing.
- Schwensow, N., Eberle, M. & Sommer, S. 2008 Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. *Proc. R. Soc. B* **275**, 555–564. (doi:10.1098/rspb.2007.1433)
- Schütz, K. E., Forkman, B. & Jensen, P. 2001 Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (*Gallus gallus*) and a modern layer strain. *Appl. Anim. Behav. Sci.* **74**, 1–14. (doi:10.1016/S0168-1591(01)00156-3)
- Shaw, I. *et al.* 2007 Different evolutionary histories of the two classical class I genes BF1 and BF2 illustrate drift and selection within the stable MHC haplotypes of chickens. *J. Immunol.* **178**, 5744–5752.
- Simmons, L. W., Beveridge, M., Wedell, N. & Tregenza, T. 2006 Postcopulatory inbreeding avoidance by female crickets only revealed by molecular markers. *Mol. Ecol.* **15**, 3817–3824. (doi:10.1111/j.1365-294X.2006.03035.x)
- Skjoldt, K., Koch, C., Crone, M. & Simonsen, M. 1985 Analysis of chickens for recombination within the Mhc (B-complex). *Tissue Antigens* **25**, 278–282.
- Sullivan, M. S. 1994 Mate choice as an information gathering process under time constraint—implications for behavior and signal-design. *Anim. Behav.* **47**, 141–151. (doi:10.1006/anbe.1994.1016)
- Thuman, K. A. & Griffith, S. C. 2005 Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Anim. Behav.* **69**, 765–770. (doi:10.1016/j.anbehav.2004.10.003)
- Tregenza, T. & Wedell, N. 2000 Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* **9**, 1013–1027. (doi:10.1046/j.1365-294x.2000.00964.x)
- Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man 1871–1971* (ed. B. Campbell), pp. 139–179. Chicago, IL: Aldine.
- Wallny, H. J. *et al.* 2006 Peptide motifs of the single dominantly expressed class I molecule explain the striking MHC-determined response to Rous sarcoma virus in chickens. *Proc. Natl Acad. Sci. USA* **103**, 1434–1439. (doi:10.1073/pnas.0507386103)
- 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. (doi:10.1098/rspb.1997.0204)
- Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J. 1995 Mhc-dependent mate preferences in humans. *Proc. R. Soc. B* **260**, 245–249. (doi:10.1098/rspb.1995.0087)
- Wedell, N., Gage, M. J. G. & Parker, G. A. 2002 Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* **17**, 313–320. (doi:10.1016/S0169-5347(02)02533-8)
- Wegner, K. M., Reusch, T. B. H. & Kalbe, M. 2003 Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *J. Evol. Biol.* **16**, 224–232. (doi:10.1046/j.1420-9101.2003.00519.x)
- Wolfinger, R. & O'Connell, M. 1993 Generalized linear mixed models—a pseudo-likelihood approach. *J. Statist. Comput. Simulation* **48**, 233–243. (doi:10.1080/00949659308811554)
- Worley, K., Gillingham, M., Jensen, P., Kennedy, L. J., Pizzari, T., Kaufman, J. & Richardson, D. S. 2008 Single locus typing of MHC class I and class IIB loci in a population of red jungle fowl. *Immunogenetics* **60**, 233–247. (doi:10.1007/s00251-008-0288-0)
- Wright, D., Kerje, S., Brändström, H., Schütz, K., Kindmark, A., Andersson, L., Jensen, P. & Pizzari, T. 2007 The genetic architecture of a female sexual ornament. *Evolution* **62**, 86–98. (doi:10.1111/j.1558-5646.2007.00281.x)
- Yamazaki, K., Boyse, E. A., Mike, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J., Zayas, Z. A. & Thomas, L. 1976 Control of mating preferences in mice by genes in major histocompatibility complex. *J. Exp. Med.* **144**, 1324–1335. (doi:10.1084/jem.144.5.1324)
- Yamazaki, K., Yamaguchi, M., Andrews, P. W., Peake, B. & Boyse, E. A. 1978 Mating preferences of F2 segregants of crosses between Mhc-congenic mouse strains. *Immunogenetics* **6**, 253–259. (doi:10.1007/BF01563915)
- 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. (doi:10.1126/science.3375818)
- Yamazaki, K., Curran, M. & Beauchamp, G. K. 1999 Fetal MHC odor types play a functional role in regulating social interactions. *Behav. Genet.* **29**, 375.
- Zeh, J. A. & Zeh, D. W. 1997 The evolution of polyandry 2: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. B* **264**, 69–75. (doi:10.1098/rspb.1997.0010)