



Original Article

Social pairing of Seychelles warblers under reduced constraints: MHC, neutral heterozygosity, and age

David J. Wright,^{a,b} Lyanne Brouwer,^c Maria-Elena Mannarelli,^b Terry Burke,^b Jan Komdeur,^d and David S. Richardson^{a,e}

^aSchool of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR4 7TJ, UK, ^bDepartment of Animal and Plant Sciences, NERC Biomolecular Analysis Facility, University of Sheffield, Sheffield S10 2TN, UK, ^cEvolution, Ecology & Genetics, Research School of Biology, The Australian National University, Canberra, Australian Capital Territory 0200, Australia, ^dBehavioural Ecology and Self-organization Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 11103, 9700 CC Groningen, The Netherlands, and ^eNature Seychelles, Centre for Environment and Education, The Sanctuary, PO Box 1310, Roche Caiman, Victoria, Mahé, Republic of Seychelles

Received 16 April 2015; revised 17 July 2015; accepted 10 August 2015; Advance Access publication 28 September 2015.

The prevalence and significance of precopulatory mate choice remains keenly debated. The major histocompatibility complex (MHC) plays a key role in vertebrate adaptive immunity, and variation at the MHC influences individual survival. Although MHC-dependent mate choice has been documented in certain species, many other studies find no such pattern. This may be, at least in part, because in natural systems constraints may reduce the choices available to individuals and prevent full expression of underlying preferences. We used translocations to previously unoccupied islands to experimentally reduce constraints on female social mate choice in the Seychelles warbler (*Acrocephalus sechellensis*), a species in which patterns of MHC-dependent extrapair paternity (EPP), but not social mate choice, have been observed. We find no evidence of MHC-dependent social mate choice in the new populations. Instead, we find that older males and males with more microsatellite heterozygosity are more likely to have successfully paired. Our data cannot resolve whether these patterns in pairing were due to male–male competition or female choice. However, our research does suggest that female Seychelles warblers do not choose social mates using MHC class I to increase fitness. It may also indicate that the MHC-dependent EPP observed in the source population is probably due to mechanisms other than female precopulatory mate choice based on MHC cues.

Key words: compatibility, extrapair paternity, good genes, MHC class I, sexual selection, translocation.

INTRODUCTION

The prevalence and significance of precopulatory mate choice remains a keenly debated topic in sexual selection, mainly due to difficulties in quantifying the evolutionary costs and benefits of being “choosy” (Andersson 1994; Kokko et al. 2003). Mate choice can provide both direct benefits, such as superior nest guarding, and indirect benefits, such as increased genetic diversity in offspring (Hamilton and Zuk 1982; Andersson 1994). It can manifest in behavioral patterns, such as the choosing of a social mate, and eventually in genetic patterns, such as bias in

offspring genotypes (Jennions and Petrie 2000; Consuegra and Garcia de Leaniz 2008). Genes of the major histocompatibility complex (MHC), which determine antigen recognition in the adaptive immune response of vertebrates (Klein 1986), have been a focus of mate choice research for decades (Yamazaki et al. 1976; Jordan and Bruford 1998; Milinski 2006; Kamiya et al. 2014). Different MHC genotypes confer differential pathogen resistance and, therefore, fitness to individuals (Briles et al. 1977; Ditchkoff et al. 2001; Wedekind et al. 2004). This makes the MHC an obvious candidate for genes that underpin the benefits of mate choice, and several hypotheses have been proposed to explain how individuals may optimize the MHC genotypes that their offspring inherits (Penn and Potts 1999; Milinski 2006; Kamiya et al. 2014).

Address correspondence to D.S. Richardson. E-mail: david.richardson@uea.ac.uk.

Various mechanisms have been invoked to explain apparent mating preferences. Individuals may choose mates based on “good genes”: either particular beneficial alleles (a classical “good genes” scenario) or on heterozygosity at specific loci (referred to as “good-genes-as-heterozygosity”), or a combination of the two. When calculated over many duplicated loci this heterozygosity can translate into choice for overall diversity, thus the latter scenario can be referred to as a “diversity” mechanism (Kamiya et al. 2014). In an MHC-dependent scenario, choice under a “good genes” or a “diversity” mechanism may be achieved by assessing indicators of condition such as secondary sexual traits that are linked to MHC characteristics (Hamilton and Zuk 1982; Ditchkoff et al. 2001; Milinski 2006). By choosing a mate with a superior MHC genotype, individuals may obtain direct benefits such as better provisioning for their offspring (Andersson 1994) as a result of the better condition (immunocompetence) of the mate or indirect benefits by providing offspring with specific advantageous alleles and/or increased MHC diversity (Hamilton and Zuk 1982; Reusch et al. 2001). Individuals may also choose mates based on their MHC similarity, in order to obtain an optimal level of MHC diversity in their offspring (Nowak et al. 1992). Mate choice under such a “compatibility” mechanism is based on indirect benefits. What constitutes a “good match” depends on the complementarity of the maternal and paternal genotypes (Yamazaki et al. 1976; Milinski 2006), although maximizing dissimilarity with a mate may not necessarily be the best strategy if there could be negative consequences of too high a level of MHC diversity (e.g., Milinski 2006; Kalbe et al. 2009). Importantly, as well as the ability to assess the MHC characteristics of others (e.g., via olfaction), “compatibility” mechanisms require self-recognition, so that individuals can gauge their compatibility with potential mates (Penn 2002; Milinski 2006). These mate choice models are normally viewed from the female perspective, though male choice is important in some systems (see Gillingham et al. 2009; Edward and Chapman 2011). Finally, MHC genes may act as markers of relatedness and be used to avoid close inbreeding, rather than to acquire specific MHC characteristics per se (Brown and Eklund 1994; Penn and Potts 1998).

Numerous studies have investigated MHC-dependent pairing and fertilization patterns providing evidence for each of the different hypothesized mechanisms in different species (e.g., Penn and Potts 1999; Kokko et al. 2003; Andersson and Simmons 2006; Milinski 2006; Kotiaho and Puurtinen 2007; Griggio et al. 2011; Løvlie et al. 2013; Kamiya et al. 2014). However, many other studies find no evidence of MHC dependence (e.g., Paterson and Pemberton 1997; Westerdahl 2004; Huchard et al. 2010), and the prevalence of MHC-dependent mate choice is unclear. Although many taxa may simply not have evolved mechanisms of MHC-dependent mate choice, its absence in a population may also be due to constraints on choice. Constraints may occur to some extent in almost all species (Arnqvist and Rowe 2005) due to factors such as social monogamy (Cohas et al. 2006) or forced pairings (Casalini et al. 2009). Significant research has focused on the consequences of constraints for the evolution of alternative mating strategies such as reproductive compensation and promiscuity (Cohas et al. 2006; Gowaty et al. 2007; Setchell and Huchard 2010), but the implications of mate choice constraints for MHC diversity in wild populations remain unclear.

Here, we take an experimental approach to investigate whether a reduction of constraints leads to the expression of MHC-dependent social mate choice in the Seychelles warbler (*Acrocephalus sechellensis*). This socially monogamous species was previously restricted to a

single island, Cousin, where the population has been at carrying capacity since 1982 (Brouwer et al. 2009). On Cousin, a combination of habitat saturation, longevity, and social fidelity is thought to severely constrain social mate choice (Richardson et al. 2005), indeed around 29% of adults never manage to obtain a breeding territory/position (Komdeur 1991). However, Seychelles warblers are highly promiscuous, with extrapair paternity (EPP) accounting for circa 40% of offspring (Richardson et al. 2001). This promiscuity is linked to MHC class I variation: females are more likely to have EPP if their social male is of low MHC diversity and do so with extrapair males that are more MHC diverse (Richardson et al. 2005). Consequently, this EPP improves the MHC diversity and thus survival of the female's offspring (Brouwer et al. 2010). Previous findings do not allow us to discern the mechanism generating these MHC-dependent fertilization patterns as they are consistent with both male–male competition—with less diverse MHC males less able to mate guard or acquire EPP—and active female choice for MHC diverse EPP males. We created an opportunity to test for MHC class I dependent social pairings under natural conditions by using translocations of Seychelles warblers to 2 new islands as part of the long-term conservation of this species (Richardson et al. 2006; Wright et al. 2014). Significantly more males than females were translocated to each island, where a large surfeit of optimal habitat meant that each male was able to establish a high-quality territory compared with those inhabited on the original island. Consequently, females had the opportunity to pair up with any one of multiple males, all with high-quality territories. Thus, we provide a relatively unconstrained arena in which to test whether specific social mating patterns occur and to assess whether such patterns may be driven by active female mate choice or other mechanisms. Given the offspring survival benefits resulting from mating with a MHC diverse male, we predict MHC class I characteristics will play an important role in any social mate choice in these populations. Under a “diversity” mechanism (i.e., good-genes-as-heterozygosity), we expect females to prefer males with high MHC diversity. Under a classical “good genes” mechanism, we would expect a link between male pair status and individual MHC class I alleles, if social mate choice is based on the presence of specific alleles—such as *Ase-ua4*, which has previously been shown to influence survival (Brouwer et al. 2010). Under a “compatibility” mechanism females would pair with maximally or optimally (Milinski 2006) MHC dissimilar males. Finally, we test whether stability of these pair bonds is MHC dependent. If “diversity” is important, males of low MHC diversity are more likely to suffer the subsequent breaking of any established pair bonds (divorce) than higher diversity males. If “compatibility” is important, pairs that divorce are expected to be more MHC similar than pairs that remain stable.

MATERIALS AND METHODS

Study populations

Each translocation was performed as outlined by Richardson et al. (2006), with all birds caught on Cousin (4°21'S 55°38'E, 0.29 km²). Translocation of existing pairs was avoided where possible, although a small number were transferred (Denis = 7, Frégate = 1, see Results for details). However, previous work (Komdeur 1996) showed that birds paired in the source population did not normally re-pair in the new populations in previous translocations. Individuals were identified with a unique combination of colored leg rings. A 25- μ L blood sample was taken from each bird by brachial venipuncture

and stored in absolute ethanol. A total of 58 birds (34 males, 24 females) were moved to Denis (3°48'S 55°40'E, 1.42 km²) in 2004 (Richardson et al. 2006) and 59 birds (36 males, 23 females) to Frégate (4°35'S 55°56'E, 2.19 km²) in 2011 (Wright et al. 2014). Each translocation was undertaken in 2 batches on different days (Denis: $n = 35$ on 30 May 2004, $n = 23$ on 12 June 2004; Frégate: $n = 22$ on 07 December 2011, $n = 37$ on 14 December 2011). All individuals were released at the same site on each island. Age at translocation (determined in reference to hatching year estimated at first capture) was classified into “young” (<2 years) and “old” (>2 years), following Brouwer et al. (2006). There was no bias in age of individuals in each batch and catching was undertaken across the entire island by multiple catching teams, covering all areas simultaneously (Richardson et al. 2006; Wright et al. 2014). Each new population was censused and monitored for breeding up to 3 months postrelease (Denis: continually from release until August 2004, Frégate: February 2012) and again after 1 year postrelease (Denis: May–August 2005, Frégate: March–May 2013).

Social mate choice

Territories were mapped on each new island. The average territory size of Seychelles warblers on Cousin is 230 m² (Brouwer et al. 2009). With at least 300 000 m² of suitable habitat on Denis (Richardson et al. 2006) and 390 000 m² on Frégate (Richardson and Hammers 2011), the spatial and habitat quality constraints that existed in the original population were greatly reduced (if not totally removed) for males released on the new islands. Individuals were located and followed for a minimum of 15 min repeatedly (ca. weekly) during the field period, during which interactions with other conspecifics were recorded. After a pair bond and territory are established, Seychelles warblers display a specific repertoire of behaviors that allows clear classification of pair status (Richardson et al. 2003). Previous studies found that although a few pairs may form and nest within days of translocation, most stable pairings only formed after 2 months of rapid switching (Komdeur 1996). Hence, social mate choice was assessed after 3 months and by multiple observations where possible. Pairings were reassessed 1 year after the initial social pairing observations. Divorce was identified when both individuals were resighted, with at least 1 individual in a new pair. In cases where one of a pair was not resighted over consecutive fieldwork periods, it was assumed that individual had died and the pairing was considered faithful until death. Any subsequent pairing of the surviving individual was not considered the result of divorce and was excluded from analysis, but we acknowledge this is a conservative measure.

Molecular analyses

Samples were genotyped at 30 microsatellite loci following Spurgin et al. (2014). We tested for deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium between loci using GENEPOP v. 4.1 (Raymond and Rousset 1995). Null allele frequencies were estimated using CERVUS v. 3.0 (Marshall et al. 1998). Three estimators of pairwise relatedness r (Queller and Goodnight 1989; Lynch and Ritland 1999; Wang 2002) were calculated in COANCESTRY v. 1.0 (Wang 2011). These 3 estimators were highly correlated (Mantel tests, all $r \geq 0.80$), and the results remained consistent regardless of the estimate used, therefore only Queller and Goodnight's r is reported. Standardized individual microsatellite heterozygosity (Hs) was calculated using the R package Genhet (Coulon 2010).

Variation at exon 3 of MHC class I (which codes for the peptide-binding region [PBR] involved in antigen recognition) was screened using reference-strand-mediated conformation analysis (RSCA) and the primer sets from Richardson and Westerdahl (2003), following the method of Worley et al. (2008). Each segregating RSCA variant corresponded to a unique 255 base pair amino acid coding sequence (of a total exon length of 274 base pairs, Richardson and Westerdahl 2003). A total of 10 MHC class I variants have been detected in the Seychelles warbler, with individuals possessing between 2 and 8 variants each, suggesting that at least 4 class I loci are amplified (Richardson and Westerdahl 2003). Although it is impossible to identify which locus each variant comes from, they are hereafter termed “alleles” for simplicity. This method does not provide a measure of locus-specific heterozygosity, but an overall estimate of MHC class I exon 3 diversity, which will correlate with heterozygosity across the amplified loci. This heterozygosity measure has shown to be an important parameter linked to fertilization patterns and survival in the Seychelles warbler (Richardson et al. 2005; Brouwer et al. 2010). Previous work by Hutchings (2009) detected no variation at MHC class II loci in the Seychelles warbler and so these loci were not assessed in the current study.

MHC-dependent mate choice may be based on only the functional differences between alleles. To address this, codons comprising the PBR were superimposed onto the Seychelles warbler sequences (see Richardson and Westerdahl 2003). However, these PBR codons were identified in humans (Bjorkman et al. 1987) and while strongly conserved across taxa, may not be completely accurate in the Seychelles warbler. Therefore, another way to determine functional differences between alleles is to compare between sites that have been identified as being under positive selection (positively selected sites [PSS]) in passerines. To identify these PSS, MHC class I exon 3 sequences from a range of passerine genera were downloaded from NCBI GenBank: *Acrocephalus* ($n = 16$), *Passer* ($n = 38$), *Parus* ($n = 64$, of which *Cyanistes* = 59), and *Carpodacus* ($n = 28$) and aligned to the Seychelles warbler ($n = 10$) in BIOEDIT v. 7.1 (Hall 1999). Three methods were employed to detect positive selection. Single likelihood ancestor counting (SLAC) and fast unbiased Bayesian approximation (FUBAR) are maximum likelihood methods that estimate the nonsynonymous to synonymous substitution rate (dN/dS ratio, ω) at each codon, the latter utilizing an Markov chain Monte Carlo approach to increase model accuracy (Kosakovsky Pond and Frost 2005; Murrell et al. 2013). The third method—the mixed effects model of evolution (MEME)—identifies episodic bouts of positive selection across an alignment by allowing ω to vary by codon and branch within the phylogeny (Murrell et al. 2012). Each method was implemented under the conservative general time reversible model and neighbor-joining tree with probabilities of <0.05 (SLAC and MEME) and posterior probability of ≥ 0.95 (FUBAR) using HYPHY (Kosakovsky Pond et al. 2005) and a web-based user interface operating on a remote cluster available at <http://www.datamonkey.org> (Delpont et al. 2010). Only codons identified by all 3 methods were accepted as putatively PSS.

Statistical analyses

Statistical analyses were performed in R v. 2.15 (R Development Core Team 2012) unless otherwise stated. Throughout, the term “pairs” denotes observed pairings, and “dyads” all other male–female pair combinations possible in each analysis. All comparisons of pairs and dyads were performed using randomization tests (Manly 1997) in MSEXCEL plug-in POPTOOLS v. 3.2 (Hood 2010). This is a useful approach to testing whether observed data

differ significantly from random expectation. In each instance detailed below, the difference between observed pairs and all other possible male–female dyad combinations was tested with analysis of variance (Anova). The data were then resampled without replacement (shuffled) and retested 10^5 times. Estimates of significance were calculated as the proportion of repetitions in which the resampled Anova F value was equal to, or exceeded, the test Anova F value. This approach was employed to control for any effect of inbreeding avoidance on social mate choice, by comparing mean relatedness (r) of pairs and dyads for each island. To investigate “good-genes-as-heterozygosity” diversity predictions, the probability of a male being paired was analyzed using a generalized linear model with a binomial error structure and logit-link function, with number of MHC alleles and standardized individual heterozygosity (Hs) as covariates, and island and age class as categorical fixed factors. Data from both islands were combined to maximize sample size, hence inclusion of “island” as a fixed factor in the model. Model fit was assessed by comparison against the null model (constant only) and Nagelkerke’s R^2 . We also tested for an interaction between individual MHC alleles and male pair status under a classical “good genes” scenario using Fisher’s Exact tests, with correction for multiple testing. MHC similarity between pairs and dyads was calculated in 2 ways. First, the proportion of alleles shared (S_{sp}), which is double the number of alleles shared between 2 individuals, divided by the sum of each individual’s alleles [$S_{sp} = 2N_{sp}/(N_x + N_y)$] (Wetton et al. 1987). Second, amino acid divergence (p -distance) was calculated between each MHC allele sequence for codons involved in the PBR and (separately) also for codons identified as putative PSS. Amino acid p -distances were calculated in MEGA v. 5.1 (Tamura et al. 2011), and the mean pairwise amino acid divergence between pairs and dyads was calculated. Comparisons of each measure of complementary (S_{sp} , PBR, and PSS) were then conducted using randomization tests as described previously. Mate choice under a compatibility mechanism may be masked if the optimal similarity between pairs lies close to that observed in random dyads. In this case, observed pairs are predicted to show less variation around this mean similarity than random dyads. Therefore, we tested variance in MHC similarity of observed pairs versus simulated dyads using the same randomization approach. The association between the MHC similarity of a pair and the likelihood of divorce was tested using Mann–Whitney–Wilcoxon tests on divorced versus faithful pairings, using each measure of MHC similarity (S_{sp} , PBR, and PSS). Finally, we tested for differences in MHC diversity between divorced and stable-pair individuals of each sex using the randomization approach.

RESULTS

All individuals were released unharmed in both translocations. Three months postrelease, 56 out of 58 birds (97%) were resighted on Denis and 50 out of 59 birds (85%) on Frégate. Annual survival of adult Seychelles warblers is high (84%; Brouwer et al. 2006), but unpaired birds are more difficult to locate than paired birds due to aggressive territoriality in the latter. All but one male individual was resighted on Denis in subsequent years, and we assumed similar survival on Frégate (indeed, 92% [54/59] of individuals were seen in the subsequent year on Frégate). For this reason, individuals not seen during the initial study period (Denis = 2 males, Frégate = 3 females and 6 males) were treated as alive and unpaired. A total of 40 pairings was confirmed in the 2 translocated populations: 40 males were considered paired and 30 unpaired. MHC data were

unavailable for 3 individuals (Denis = 2, Frégate = 1), and these were excluded from analyses along with their pair bird, leaving 37 pairings (Denis = 19, Frégate = 18). Eight existing pairs from Cousin were translocated (Denis = 7, Frégate = 1), but only 2 of these pairs re-paired once released (both on Denis). Observations of pairs 1 year after revealed that 29 pairs had remained together and 8 had divorced.

Genetic markers

Microsatellite and MHC class I genotypes were compiled for all 111 individuals included in the analyses (Denis = 54, Frégate = 57). Neither population showed significant departure from Hardy–Weinberg equilibrium at any microsatellite locus. Linkage disequilibrium was detected between 1 loci pair in Denis (*Ase-13* and *Ase-48*, $P_{crit} = 0.0001$) and between 2 loci pairs in Frégate (*Ase-56* and *Ase-38*, *Ase-48* and *Cuu4-gga*, both $P_{crit} = 0.0001$) after Bonferroni correction. Null allele frequencies of 0.11 were detected at loci *Ase-56* and *Ase-38* in the Frégate population, but -0.013 and -0.096 , respectively, in the Denis population. Analyses repeated with and without one of each pair of loci showed no qualitative difference (data not shown) and so all loci were retained.

There was no difference in the number of MHC class I alleles in each sex (females: $n = 44$, mean \pm standard error [SE] = 4.70 ± 0.22 vs. males: $n = 67$, mean \pm SE = 4.61 ± 0.17 , $W = 1462.5$, $P = 0.94$) or between islands (Denis = 4.77 ± 0.18 vs. Frégate = 4.52 ± 0.20 , $W = 1362$, $P = 0.28$), and all 10 alleles were present in each population. Selection tests indicated that 7 of the 85 codons of exon 3 were putatively PSS, of which 3 corresponded to the 7th, 9th, and 11th codons of the superimposed PBR (Supplementary Figure S1).

Social mate choice

Inbreeding avoidance

Pairwise relatedness (r) of social pairs varied between $r = -0.31$ and 0.41 (mean \pm SE = 0.02 ± 0.03) across both populations with 5 pairs being related to the degree of half-sib or higher ($r > 0.25$). No difference was detected between the relatedness of pairs versus random dyads on either island (randomization tests: Denis 19 pairs vs. 342 dyads, $P = 0.40$ and Frégate 18 pairs vs. 306 dyads, $P = 0.16$).

Good-genes-as-heterozygosity

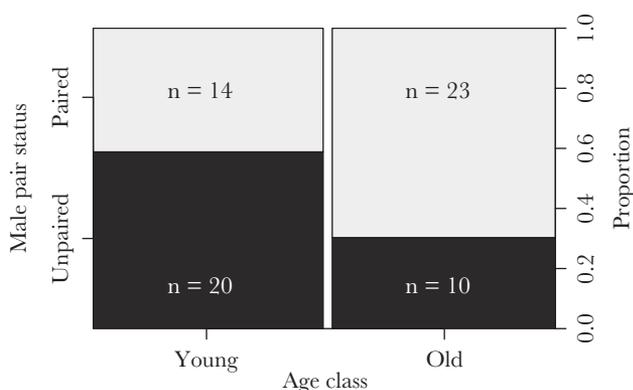
The full generalized linear model (Table 1) was a significant improvement on the null model ($\chi^2 = 10.59$, degrees of freedom = 4, $P = 0.03$). No collinearity was detected between predictors (all tolerances ≥ 0.88). MHC diversity did not predict whether males were paired or not (Table 1), with a mean (\pm SE) MHC diversity of paired males = 4.49 ± 0.24 and unpaired males = 4.77 ± 0.25 . Age class and, to a lesser degree, individual neutral heterozygosity, were both significant predictors of male pair status (Table 1). Older males ($n = 33$) were more likely to be paired than younger males ($n = 34$, 69.7% vs. 41.2%, respectively, Figure 1) and paired males ($n = 37$) were generally more heterozygous than unpaired males ($n = 30$, mean \pm SE of 1.04 ± 0.03 vs. 0.95 ± 0.03 , respectively, Welch’s $t_{64.83} = 1.94$, $P = 0.05$, Figure 2), but this was not due to older males being more heterozygous (older males mean \pm SE = 1.00 ± 0.03 vs. young males = 1.00 ± 0.03 ; Welch’s $t_{64.83} = 0.02$, $P = 0.98$). Similarly, MHC diversity did not differ between male age categories (older males mean \pm SE = 4.54 ± 0.24 vs. young males = 4.68 ± 0.25 , $W = 563$, $P = 0.98$). Including which transfer batch (date) individuals were moved, or testing for a quadratic effect of male MHC diversity did not significantly

Table 1

Generalized linear model with a binomial error structure and logit-link function, predicting the pairing status of male Seychelles warbler within the newly established populations in relation to MHC class I diversity, age class (young or old), individual standardized heterozygosity (Hs), and island (Denis or Frégate)

Predictors	<i>B</i> (SE)	χ^2	df	95% CI for odds ratio			<i>P</i> value
				Lower	Odds ratio	Upper	
Constant	-2.49 (1.72)	-1.45	1				
MHC diversity	-0.19 (0.19)	-0.98	1	0.56	0.83	1.2	0.32
Age class	1.27 (0.57)	2.23	1	1.2	3.55	11.38	0.02
Hs	2.95 (1.46)	2.02	1	1.23	19.16	413.1	0.04
Island	0.03 (0.56)	0.05	1	0.34	1.03	3.22	0.96

Maximum model $\chi^2 = 10.59$, $df = 4$, $P = 0.03$, and $R^2 = 0.20$ (Nagelkerke). Parameters significant at $P < 0.05$ are highlighted in bold. CI, confidence interval; df = degrees of freedom.

**Figure 1**

Proportions of male Seychelles warblers paired (light gray) and unpaired (dark gray) in 2 newly established populations for each age class, 3-month postrelease: young (<2 years, $n = 34$) and old (>2 years, $n = 33$). Data are combined from both translocated populations.

increase the explanatory power of the model and did not influence the effects of age and microsatellite heterozygosity on male pairing.

Classical good genes

Fisher's Exact tests showed 1 allele, *Ase-ua3*, had a weak negative relationship with male pair status (i.e., the presence of *Ase-ua3* meant lower probability of being paired) before correction for multiple testing ($P = 0.03$, odds ratio: 0.26), but there were no significant interactions between any of the individual MHC class I alleles and male pair status after Bonferroni correction for multiple testing ($P_{crit} = 0.005$).

MHC compatibility

There was no difference in mean MHC allele sharing (S_y) between pairs ($n = 19$) and random dyads ($n = 342$) on Denis (mean \pm standard deviation: pairs = 0.64 ± 0.30 vs. dyads = 0.64 ± 0.25 , randomization test $P = 0.95$) or between pairs ($n = 18$) and random dyads ($n = 306$) on Frégate (pairs = 0.58 ± 0.28 vs. dyads = 0.50 ± 0.28 , $P = 0.22$). Furthermore, there was no difference in the variance between pairs and random dyads (Denis $P = 0.87$, Frégate $P = 0.28$, details above). Similarly, there was no difference in mean amino acid divergence (p -distance) between pairs versus random dyads for either the PBR codons (Denis pairs = 0.36 ± 0.026 vs. dyads = 0.36 ± 0.031 , randomization tests $P = 0.69$ and Frégate pairs = 0.33 ± 0.05 vs. dyads = 0.35 ± 0.04 , $P = 0.19$) or the PSS codons (Denis pairs = 0.38 ± 0.037 vs. dyads = 0.38 ± 0.039 ,

$P = 0.89$ and Frégate pairs = 0.34 ± 0.07 vs. dyads = 0.36 ± 0.06 , $P = 0.14$).

Divorce

Males that divorced ($n = 8$) within 1 year after translocation had lower MHC diversity than males of stable ($n = 29$) pairs (mean \pm SE: divorced = 3.37 ± 0.46 vs. stable = 4.79 ± 0.25 , randomization test $P = 0.02$). However, there was no difference in MHC diversity of stable or divorced females (divorced = 4.13 ± 0.74 vs. stable = 4.79 ± 0.25 , randomization test $P = 0.30$). Similarly, no difference was detected between divorced versus stable pairs with any of the measures of MHC similarity: S_y (divorced = 0.59 ± 0.10 vs. stable = 0.61 ± 0.05 , $W = 128$, $P = 0.67$), PBR p -distance (divorced = 0.32 ± 0.02 vs. stable = 0.35 ± 0.01 , $W = 165.5$, $P = 0.10$), or PSS p -distance (divorced = 0.30 ± 0.03 vs. stable = 0.37 ± 0.01 , $W = 161.5$, $P = 0.07$).

DISCUSSION

We found no evidence that the occurrence of social pairings in founding populations of Seychelles warblers was influenced by MHC class I characteristics, that is, male MHC diversity, specific alleles, or male–female MHC compatibility within the new island populations. However, older and more neutrally heterozygous males were more likely to be paired. Even when constraints on female choice were greatly reduced, the MHC-dependent patterns of extrapair fertilizations observed in the original source population did not translate into MHC-dependent social mate choice in the new populations. Furthermore, we show that the relatedness of 5 pairs actually exceeded half-sibship ($r > 0.25$) suggesting little attempt or ability even to avoid inbreeding, a result consistent with previous work on the source population (Richardson et al. 2004; Eikenaar et al. 2008).

Divorced males appeared to be less MHC diverse than those in stable pairings. This pattern is consistent with the previous work on MHC-dependent EPP (Richardson et al. 2005) in which less MHC diverse males were cuckolded by more MHC diverse males. The small sample size (8 divorced, 29 stable) means it should be interpreted with caution and we do not possess data on the new pairings of divorced birds for comparison. These data do not enable us to distinguish between active female choice to leave, or male–male competition/male condition, where a male might be less able to defend a mate or territory from a rival (e.g., Hasselquist and Sherman 2001). It does, however, point to a possible link between pair stability and male MHC diversity worthy of future investigation. A slight trend was observed between divorce and MHC PSS

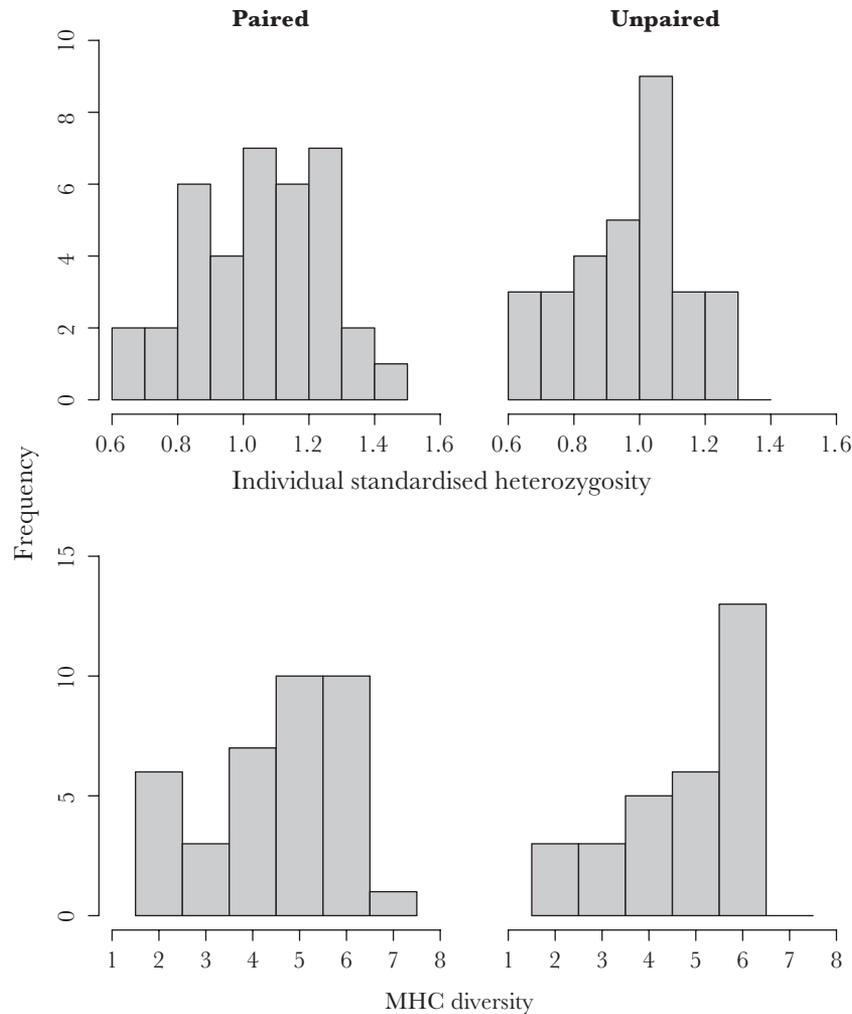


Figure 2

Frequency histograms of individual standardized heterozygosity (upper) and MHC class I diversity (lower) in paired ($n = 37$) and unpaired ($n = 30$) male Seychelles warblers in the newly established populations 3-month postrelease.

($P = 0.07$) that suggests pairs with more similarity at PSS were more likely to divorce than those more different at PSS. Although this is intriguing, we acknowledge that this may just be a spurious tendency and statistical power and further inference is limited.

The lack of an association between MHC class I diversity or specific alleles and social mate choice suggests that MHC-dependent social mate choice does not occur in this species, a result that concurs with that observed in the congeneric great reed warbler (*Acrocephalus arundinaceus*) (Westerdahl 2004) and other passerines such as the great tit (*Parus major*) (Sepil et al. 2015). The importance of recognition mechanisms such as olfaction in avian reproductive behavior is now widely acknowledged (Caro et al. 2015), and MHC-dependent mate choice suggestive of an olfactory capability has been reported in blue petrels (*Halobaena caerulea*) (Strandh et al. 2012). However, to our knowledge, a mechanism allowing self-recognition and assessment of MHC compatibility has not yet been reported in passerines. Alternatively, subtle patterns of MHC-dependent social mate choice may not have been detected owing to a limited sample size ($n = 37$ pairs). However, the complete distribution overlap of MHC diversity of paired and unpaired males within the dataset (Figure 2) suggests the lack of a significant result is not due to a type 2 error, and similar samples sizes have detected

MHC-dependent patterns in other studies of wild populations (Bonneau et al. 2006; Juola and Dearborn 2012). The accuracy of likelihood selection methods (Anisimova et al. 2003) and thus the identification of PSS could have been reduced by recombination because complex recombination (e.g., gene conversion) can occur at the MHC region (Witzell et al. 1999; Spurgin et al. 2011). Therefore, we acknowledge that the conservative tests employed here could have misidentified or missed PSS thus weakening our ability to detect choice based on these sites. However, because our previous work found associations with MHC class I diversity and fertilization, we suggest that the absence of MHC-dependent social mate choice, even when choice constraints have been much reduced, is the most likely explanation of our findings.

Older and more heterozygous males were more likely to be paired than younger and less heterozygous males, but this was not due to more heterozygous males being older. The finding that heterozygous males were more likely to be paired could be explained by mate choice under a “good-genes-as-heterozygosity” diversity model, which has been reported in birds (Kempnaers 2007). The presence of closely related pairs in the new populations suggests that male–female complementarity does not play a role in this. Indeed, as Seychelles warbler pair bonds last over multiple breeding

attempts, overall male quality (i.e., heterozygosity) may be a more important factor than diversity at specific genes. Age-dependent social mate choice (Kokko and Lindstrom 1996; Kokko 1998) and extrapair fertilization success (Richardson and Burke 1999; Cleasby and Nakagawa 2012) have both been observed in various species. Even though Seychelles warblers can breed successfully at 8 months of age (Komdeur 1992), territory acquisition is age related, with older males more likely to gain a breeding territory than younger ones, probably mediated by male–male competition (Eikenaar et al. 2009). However, there was a surfeit of quality habitat in our translocated populations, with none of the spatial constraints present on Cousin, as the new islands were unoccupied by Seychelles warblers at the time of translocation. Active female mate choice is only part of sexual selection and mechanisms such as male–male competition can skew social mate choice patterns, whereas sperm competition and cryptic female choice may bias fertilization patterns (Andersson 1994; Jennions and Petrie 2000; Kotiaho and Puurtinen 2007; Løvlie et al. 2013). It is plausible that older or more heterozygous males were more successfully able to compete for females, perhaps through other forms of male–male competition or aggressive coercion (Casalini et al. 2009). Indeed, competitive ability is thought to increase with age in many species (Shutler and Weatherhead 1991; Bose and Sarrazin 2007; Laskemoen et al. 2008). However, our observations showed that males occupied territories and were singing to attract females. Furthermore, females can switch partners readily and initial pairings appear to take time (Komdeur 1996), indicating both a “choosing” period and lack of forced coercion by males. Nevertheless, we cannot rule out that our findings are a result of male–male competition.

Negative results of mate choice studies are generally unlikely to be published (Bernatchez and Landry 2003; Kotiaho and Puurtinen 2007), but such findings are important in establishing the extent to which active mate choice for functional genes such as the MHC occurs. The results of our study suggest that random social pairing with respect to MHC class I characteristics occurs in the Seychelles warbler, regardless of whether or not constraints are present. The occurrence of MHC-dependent EPP (Richardson et al. 2005) suggests an interaction between MHC genes and fertilization patterns that is important in maintaining MHC diversity in this species. However, it may be that the historical constraints on, and costs associated with, social mate choice preferences (Kokko et al. 2003) have prevented the evolution of MHC-dependent social mate choice in the Seychelles warbler, with alternative mechanisms such as age-dependent pairing, promiscuity (driven by whatever mechanism), or postcopulatory selection (e.g., Løvlie et al. 2013) taking precedence. This study highlights that predicting the occurrence of a sexual selection mechanism, that is, MHC-dependent social mate choice, based on seemingly related observations, that is, the MHC-dependent EPP patterns and survival, is not straightforward and that the EPP pattern (Richardson et al. 2005) may reflect mechanisms other than active female choice. There are many potential sexual selection mechanisms that may evolve separately or in concert such as precopulatory or postcopulatory mate choice, coercion, or promiscuity (Andersson 1994; Andersson and Simmons 2006). Understanding how, when, and why particular mechanisms evolve, while others do not, or are observable, while others are not, requires an understanding of the constraints acting on any given species or population. We encourage future studies to focus on how patterns and mechanisms linked to sexual selection may be influenced by the constraints acting within a system.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

FUNDING

The work was supported by a Natural Environment Research Council (NERC) PhD CASE studentship with BirdLife International to D.J.W. supervised by D.S.R. and by NERC grants to D.S.R. (NER/I/S/2002/00712 and NE/F02083X/1). L.B. was supported by an Australian Research Council DECRA fellowship (DE130100174).

We thank Nature Seychelles for facilitating the Seychelles warbler project and allowing us to work on Cousin, and the proprietors of Denis and Frigate for supporting the translocations and for access to the islands. We thank L. Spurgin, K. Phillips, and J. Gill for advice on analyses, NERC Biomolecular Analysis Facility at Sheffield University for facilities to run the RSCA analysis, M. van der Velde for microsatellite laboratory work, and the many field assistants for help with various aspects of the project. The Seychelles Bureau of Standards and the Department of Environment gave permission for sampling and fieldwork, and all work was approved by the UEA animal welfare and ethical review body.

Data accessibility: MHC sequence data are available under their GenBank accession numbers. Original data are available from the corresponding author, david.richardson@uea.ac.uk.

Handling editor: Shinichi Nakagawa

REFERENCES

- Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Andersson M, Simmons LW. 2006. Sexual selection and mate choice. *Trends Ecol Evol.* 21:296–302.
- Anisimova M, Nielsen R, Yang Z. 2003. Effect of recombination on the accuracy of the likelihood method for detecting positive selection at amino acid sites. *Genetics.* 164:1229–1236.
- Arnqvist G, Rowe L. 2005. Sexual conflict. Princeton (NJ): Princeton University Press.
- Bernatchez L, Landry C. 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? *J Evol Biol.* 16:363–377.
- Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. 1987. Structure of the human class-I histocompatibility antigen, HLA-A2. *Nature.* 329:506–512.
- Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G. 2006. Complex Mhc-based mate choice in a wild passerine. *Proc Biol Sci.* 273:1111–1116.
- Bose M, Sarrazin F. 2007. Competitive behaviour and feeding rate in a reintroduced population of griffon vultures *Gyps fulvus*. *Ibis.* 149:490–501.
- Briles WE, Stone HA, Cole RK. 1977. Mareks-disease—effects of B-histocompatibility alloalleles in resistant and susceptible chicken lines. *Science.* 195:193–195.
- 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.* 19:3444–3455.
- Brouwer L, Richardson DS, Eikenaar C, Komdeur J. 2006. The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine. *J Anim Ecol.* 75:1321–1329.
- Brouwer L, Tinbergen JM, Both C, Bristol R, Richardson DS, Komdeur J. 2009. Experimental evidence for density-dependent reproduction in a cooperatively breeding passerine. *Ecology.* 90:729–741.
- Brown JL, Eklund A. 1994. Kin recognition and the major histocompatibility complex—an integrative review. *Am Nat.* 143:435–461.
- Caro SP, Balthazart J, Bonadonna F. 2015. The perfume of reproduction in birds: chemosignaling in avian social life. *Horm Behav.* 68:25–42.

- Casalini M, Agbali M, Reichard M, Konecna M, Bryjova A, Smith C. 2009. Male dominance, female mate choice and intersexual conflict in the rose bitterling (*Rhodeus ocellatus*). *Evolution*. 63:366–376.
- Cleasby IR, Nakagawa S. 2012. The influence of male age on within-pair and extra-pair paternity in passerines. *Ibis*. 154:318–324.
- Cohas A, Yoccoz NG, Silva A, Goossens B, Allainé D. 2006. Extra-pair paternity in the monogamous alpine marmot (*Marmota marmota*): the roles of social setting and female mate choice. *Behav Ecol Sociobiol*. 59:597–605.
- Consuegra S, Garcia de Leaniz C. 2008. MHC-mediated mate choice increases parasite resistance in salmon. *Proc Biol Sci*. 275:1397–1403.
- Coulon A. 2010. GENHET: an easy-to-use R function to estimate individual heterozygosity. *Mol Ecol Resour*. 10:167–169.
- Delpont W, Poon AF, Frost SD, Kosakovsky Pond SL. 2010. Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics*. 26:2455–2457.
- Ditchkoff SS, Lochmiller RL, Masters RE, Hooper SR, Van Den Bussche RA. 2001. Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (*Odocoileus virginianus*): evidence for good-genes advertisement. *Evolution*. 55:616–625.
- Edward DA, Chapman T. 2011. The evolution and significance of male mate choice. *Trends Ecol Evol*. 26:647–654.
- Eikenaar C, Komdeur J, Richardson DS. 2008. Natal dispersal patterns are not associated with inbreeding avoidance in the Seychelles warbler. *J Evol Biol*. 21:1106–1116.
- Eikenaar C, Richardson DS, Brouwer L, Bristol R, Komdeur J. 2009. Experimental evaluation of sex differences in territory acquisition in a cooperatively breeding bird. *Behav Ecol*. 20:207–214.
- Gillingham MA, Richardson DS, Løvlie H, Moynihan A, Worley K, Pizzari T. 2009. Cryptic preference for MHC-dissimilar females in male red junglefowl, *Gallus gallus*. *Proc Biol Sci*. 276:1083–1092.
- Gowaty PA, Anderson WW, Bluhm CK, Drickamer LC, Kim YK, Moore AJ. 2007. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. *Proc Natl Acad Sci USA*. 104:15023–15027.
- Griggio M, Biard C, Penn DJ, Hoi H. 2011. Female house sparrows “count on” male genes: experimental evidence for MHC-dependent mate preference in birds. *BMC Evol Biol*. 11:44.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*. 41:95–98.
- Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds—a role for parasites. *Science*. 218:384–387.
- Hasselquist D, Sherman PW. 2001. Social mating systems and extrapair fertilizations in passerine birds. *Behav Ecol*. 12:457–466.
- Hood GM. 2010. PopTools version 3.2.5 [Internet]. Available from: <http://www.poptools.org>.
- Huchard E, Knapp LA, Wang J, Raymond M, Cowlshaw G. 2010. MHC, mate choice and heterozygote advantage in a wild social primate. *Mol Ecol*. 19:2545–2561.
- Hutchings K. 2009. Parasite-mediated selection in an island endemic, the Seychelles warbler (*Acrocephalus sechellensis*). Norwich (UK): University of East Anglia. p. 170.
- Jennions MD, Petrie M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol Rev Camb Philos Soc*. 75:21–64.
- Jordan WC, Bruford MW. 1998. New perspectives on mate choice and the MHC. *Hereditas (Edinb)*. 81(Pt 3):239–245.
- Juola FA, Dearborn DC. 2012. Sequence-based evidence for major histocompatibility complex-disassortative mating in a colonial seabird. *Proc Biol Sci*. 279:153–162.
- Kalbe M, Eizaguirre C, Dankert I, Reusch TB, Sommerfeld RD, Wegner KM, Milinski M. 2009. Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proc Biol Sci*. 276:925–934.
- Kamiya T, O'Dwyer K, Westerdahl H, Senior A, Nakagawa S. 2014. A quantitative review of MHC-based mating preference: the role of diversity and dissimilarity. *Mol Ecol*. 23:5151–5163.
- Kempenaers B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. In: Brockmann HJ, Roper TJ, Naguib M, Wynne-Edwards KE, Barnard C, Mitani J, editors. *Advances in the study of behavior*. Vol. 37. p. 189–278.
- Klein J. 1986. *Natural history of the major histocompatibility complex*. New York: John Wiley & Sons.
- Kokko H. 1998. Good genes, old age and life-history trade-offs. *Evol Ecol*. 12:739–750.
- Kokko H, Brooks R, Jennions MD, Morley J. 2003. The evolution of mate choice and mating biases. *Proc Biol Sci*. 270:653–664.
- Kokko H, Lindstrom J. 1996. Evolution of female preference for old mates. *Proc Biol Sci*. 263:1533–1538.
- Komdeur J. 1991. *Cooperative breeding in the Seychelles warbler*. Cambridge (UK): University of Cambridge.
- Komdeur J. 1992. Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature*. 358:493–495.
- Komdeur J. 1996. Influence of helping and breeding experience on reproductive performance in the Seychelles warbler: a translocation experiment. *Behav Ecol*. 7:326–333.
- Kosakovsky Pond SL, Frost SD. 2005. Not so different after all: a comparison of methods for detecting amino acid sites under selection. *Mol Biol Evol*. 22:1208–1222.
- Kosakovsky Pond SL, Frost SDW, Muse SV. 2005. HyPhy: hypothesis testing using phylogenies. *Bioinformatics*. 21:676–679.
- Kotiaho JS, Puurtinen M. 2007. Mate choice for indirect genetic benefits: scrutiny of the current paradigm. *Funct Ecol*. 21:638–644.
- Laskemoen T, Fossoy F, Rudolfen G, Lifjeld JT. 2008. Age-related variation in primary sexual characters in a passerine with male age-related fertilization success, the bluethroat *Luscinia svecica*. *J Avian Biol*. 39:322–328.
- Lieutenant-Gosselin M, Bernatchez L. 2006. Local heterozygosity-fitness correlations with global positive effects on fitness in threespine stickleback. *Evolution*. 60:1658–1668.
- Løvlie H, Gillingham MA, Worley K, Pizzari T, Richardson DS. 2013. Cryptic female choice favours sperm from major histocompatibility complex-dissimilar males. *Proc Biol Sci*. 280:20131296.
- Lynch M, Ritland K. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics*. 152:1753–1766.
- Manly BJF. 1997. *Randomization, bootstrap and Monte Carlo methods in biology*. London: Chapman and Hall.
- Marshall TC, Slate J, Kruuk LE, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol*. 7:639–655.
- Milinski M. 2006. The major histocompatibility complex, sexual selection, and mate choice. *Ann Rev Ecol Syst*. 37:159–186.
- Murrell B, Moola S, Mabona A, Weighill T, Sheward D, Kosakovsky Pond SL, Scheffler K. 2013. FUBAR: a fast, unconstrained Bayesian approximation for inferring selection. *Mol Biol Evol*. 30:1196–1205.
- Murrell B, Wertheim JO, Moola S, Weighill T, Scheffler K, Kosakovsky Pond SL. 2012. Detecting individual sites subject to episodic diversifying selection. *PLoS Genet*. 8:e1002764.
- Nowak MA, Tarczy-Hornoch K, Austyn JM. 1992. The optimal number of major histocompatibility complex molecules in an individual. *Proc Natl Acad Sci USA*. 89:10896–10899.
- Paterson S, Pemberton JM. 1997. No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proc Biol Sci*. 264:1813–1819.
- Penn DJ. 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*. 108:1–21.
- Penn D, Potts W. 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proc Biol Sci*. 265:1299–1306.
- Penn DJ, Potts WK. 1999. The evolution of mating preferences and major histocompatibility complex genes. *Am Nat*. 153:145–164.
- Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. *Evolution*. 43:258–275.
- R Development Core Team. 2012. *R: a language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>.
- Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered*. 86:248–249.
- Reusch TB, Häberli MA, Aeschlimann PB, Milinski M. 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*. 414:300–302.
- Richardson DS, Bristol R, Shah NJ. 2006. Translocation of the Seychelles warbler *Acrocephalus sechellensis* to establish a new population on Denis Island, Seychelles. *Conserv Evid*. 3:54–57.
- Richardson DS, Burke T. 1999. Extra-pair paternity in relation to male age in Bullock's orioles. *Mol Ecol*. 8:2115–2126.
- Richardson DS, Burke T, Komdeurs J. 2003. Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. *J Evol Biol*. 16:854–861.

- Richardson DS, Hammers M. 2011. Assessment of the suitability of Frégate Island for Seychelles warblers (*Acrocephalus sechellensis*). Norwich (UK): University of East Anglia Report.
- Richardson DS, Jury FL, Blaakmeer K, Komdeur J, Burke T. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). *Mol Ecol*. 10:2263–2273.
- Richardson DS, Komdeur J, Burke T. 2004. Inbreeding in the Seychelles warbler: environment-dependent maternal effects. *Evolution*. 58:2037–2048.
- 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 Biol Sci*. 272:759–767.
- Richardson DS, Westerdahl H. 2003. MHC diversity in two *Acrocephalus* species: the outbred great reed warbler and the inbred Seychelles warbler. *Mol Ecol*. 12:3523–3529.
- Sepil I, Radersma R, Santure AW, De Cauwer I, Slate J, Sheldon BC. 2015. No evidence for MHC class I-based disassortative mating in a wild population of great tits. *J Evol Biol*. 28:642–654.
- Setchell JM, Huchard E. 2010. The hidden benefits of sex: evidence for MHC-associated mate choice in primate societies. *Bioessays*. 32:940–948.
- Shutler D, Weatherhead PJ. 1991. Owner and floater red-winged blackbirds—determinants of status. *Behav Ecol Sociobiol*. 28:235–241.
- Spurgin LG, van Oosterhout C, Illera JC, Bridgett S, Gharbi K, Emerson BC, Richardson DS. 2011. Gene conversion rapidly generates major histocompatibility complex diversity in recently founded bird populations. *Mol Ecol*. 20:5213–5225.
- Spurgin LG, Wright DJ, van der Velde M, Collar NJ, Komdeur J, Burke T, Richardson DS. 2014. Museum DNA reveals the demographic history of the endangered Seychelles warbler. *Evol Appl*. 7:1134–1143.
- Strandh M, Westerdahl H, Pontarp M, Canbäck B, Dubois MP, Miquel C, Taberlet P, Bonadonna F. 2012. Major histocompatibility complex class II compatibility, but not class I, predicts mate choice in a bird with highly developed olfaction. *Proc Biol Sci*. 279:4457–4463.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 28:2731–2739.
- Wang J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics*. 160:1203–1215.
- Wang J. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol Ecol Resour*. 11:141–145.
- Wedekind C, Walker M, Portmann J, Cenni B, Müller R, Binz T. 2004. MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *J Evol Biol*. 17:11–18.
- Westerdahl H. 2004. No evidence of an MHC-based female mating preference in great reed warblers. *Mol Ecol*. 13:2465–2470.
- Wetton JH, Carter RE, Parkin DT, Walters D. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*. 327:147–149.
- Witzell H, Bernot A, Auffray C, Zoorob R. 1999. Concerted evolution of two Mhc class II B loci in pheasants and domestic chickens. *Mol Biol Evol*. 16:479–490.
- Worley K, Gillingham M, Jensen P, Kennedy LJ, Pizzari T, Kaufman J, Richardson DS. 2008. Single locus typing of MHC class I and class II B loci in a population of red jungle fowl. *Immunogenetics*. 60:233–247.
- Wright DJ, Shah NJ, Richardson DS. 2014. Translocation of the Seychelles warbler *Acrocephalus sechellensis* to establish a new population on Frégate Island, Seychelles. *Conserv Evid*. 11:20–24.
- Yamazaki K, Boyse EA, Mike V, Thaler HT, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, Thomas L. 1976. Control of mating preferences in mice by genes in major histocompatibility complex. *J Exp Med*. 144:1324–1335.