No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population

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SUMMARY

Conventionally, the extraordinary diversity of the vertebrate major histocompatibility complex (MHC) is thought to have evolved in response to parasites and pathogens affecting fitness. More recently, reproductive mechanisms such as disassortative mating have been suggested as alternative mechanisms maintaining MHC diversity. A large unmanaged population of Soay sheep (*Ovis aries* L.) was used to investigate reproductive mechanisms in the maintenance of MHC diversity. Animals were sampled as new-born lambs and between 887 and 1209 individuals were typed at each of five microsatellite markers located either within or flanking the ovine MHC. All loci were in Hardy–Weinberg proportions. A novel likelihood-based approach was developed to analyse mating patterns using paternity data. No evidence for non-random mating with respect to MHC markers was found using this technique. We conclude that MHC diversity in the St Kildan Soay sheep population is unlikely to be maintained by mating preferences and that, in contrast with evidence from experimental mice populations, MHC variation plays no role in the mating structure of this population.

1. INTRODUCTION

The major histocompatibility complex (MHC) of vertebrates encodes a group of closely linked genes involved in antigen presentation to the immune system. High levels of diversity and polymorphism at certain loci within the complex have been documented in a wide range of vertebrate species (Klein 1986) and many lines of evidence indicate a selective force behind MHC diversity. First, for given sample sizes and numbers of alleles, the allele frequencies at MHC loci show a very even distribution, leading to higher levels of heterozygosity than can be explained under neutral theory (Hedrick & Thomson 1983). Second, the rate of non-synonymous (coding) substitution exceeds the rate of synonymous (non-coding) substitution, especially at the antigen presenting site (APS), favouring new MHC variants and increasing diversity (Hughes & Nei 1988; Hughes & Nei 1989). Finally, when genealogies of MHC alleles are constructed it is frequently observed that the divergence of allelic MHC lineages predate the speciation event giving rise to separate taxa, indicating the action of balancing selection over long periods of evolutionary time (Klein *et al*. 1993; Yuhki & O'Brien 1997).

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Due to the central role of the MHC in the vertebrate immune system, it is widely held that parasites play a major part in the maintenance of MHC diversity through selective effects upon their hosts (Ebert & Hamilton 1996; Hill *et al*. 1991), maintaining MHC diversity through either frequency-dependent selection or overdominance. MHC variation has been associated with parasite resistance in a number of studies involving human, livestock and laboratory populations (Briles *et al*. 1977; Hill *et al*. 1991; Keymer *et al*. 1990; Schwaiger *et al*. 1995). However, the role of the MHC can also be considered in terms of self/non-self recognition. Although this aspect of MHC function is generally viewed in terms of pathogen presentation and response, the MHC may play a significant role in intraspecific interactions (Potts & Wakeland 1990).

It has been proposed that either mating preferences or selective abortion on the basis of MHC type can lead to increased MHC diversity and heterozygosity and may act as a means of avoiding inbreeding. After excluding other mechanisms, Potts *et al*. (1991) concluded that MHC-based mate-choice occurred in a semi-natural mouse population, in which females showed preferences for territories of males with dissimilar MHC genotypes. Such preferences may arise from MHC-derived odour profiles in male mice (Singh *et al*. 1987). Similar work on human subjects suggest that women can show preferences for body odours from

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MHC-dissimilar males (Wedekind *et al*. 1995). Recent experiments by Singer *et al*. (1997) suggest that the relative proportions of various volatile carboxylic acids may be an important determinant of MHC-based odour differences in mice urine. In further studies of a semi-natural mouse population, Potts *et al*. (1994) were able to demonstrate that MHC-based female preferences increased offspring fitness by reducing inbreeding with close relatives rather than by reducing offspring pathogen load.

To establish the importance of MHC-dependent reproductive mechanisms, such as mating preferences, in the evolution of MHC diversity, it would be valuable to have studies of several natural populations involving a range of vertebrate species. Our analysis is based upon a large, unmanaged, individually monitored population of Soay sheep on St Kilda, Scotland (Campbell 1974; Clutton-Brock *et al*. 1991, 1992). Evidence for balancing selection at the MHC has been found in this population. First, two microsatellite markers within the MHC, although in Hardy–Weinberg proportions, have unexpectedly even allele frequencies, and second, coding variation in an expressed class II gene shows an excess of non-synonymous over synonymous substitutions, implying selection for expressed MHC diversity over evolutionary time (Paterson 1998). In this paper, using known mother–offspring pairs, paternal identities inferred by genetic methods and a novel statistical technique for examining patterns of mate-choice on the basis of MHC genotype, we find no evidence for MHC-dependent mating patterns in the Soay sheep population.

2. MATERIALS AND METHODS

(*a*) *Study site and animals*

The archipelago of St Kilda lies 45 miles west of the Outer Hebrides, Scotland, at 57 °49′N, 08 °34′W, and consists of the islands of Hirta, Soay, Dun and Boreray. The Soay sheep is a primitive breed of sheep, resembling the Neolithic domestic sheep first brought to Britain *ca*. 5000 BC. It may have been introduced to St Kilda as early as *ca*. 2000 BC, but in historic times has been restricted to the uninhabited island of Soay (Campbell 1974). In 1932, following the evacuation of the human population two years previously, 107 Soay sheep (20 rams, 44 ewes, 21 ewe lambs and 22 castrated ram lambs) were introduced from Soay (99 Ha) to the larger island of Hirta (638 Ha) (Campbell 1974). Numbers increased rapidly and since 1952 have fluctuated between 600 and 1800 animals. The population of sheep within the Village Bay study area (*ca*. 200 Ha) represents around one-third of the total Hirta population, fluctuating in size between 200 and 550 animals and correlating closely with the remaining island population (Clutton-Brock *et al*. 1991, 1992). Since 1985, at least 90% of lambs born within the study area have been caught and individually tagged within a few days of birth. Blood samples and ear punches for genetic analysis are taken from lambs at this time.

(*b*) *Mating system*

The mating structure of the Soay sheep population has been documented by Grubb (1974) and is the subject of ongoing research. Soay females are highly seasonal and synchronous, typically coming into oestrus in November when there is thus a competitive rut. Most females conceive and produce one or two lambs five months later in April. In common with many male mammals, Soay males are polygynous. Males do not defend territories or harems of females, but instead attempt to form exclusive consorts with single oestrous females and mate repeatedly with them. Consorting males are frequently supplanted by dominants. Furthermore, if no single male is dominant enough to defend an oestrous female against other males, multimale chases result, at the end of which it is common for several of the chasing males to mate with the chased female. The extent to which either sex chooses a mate is not obvious, but it is possible, for example, that females instigate confrontations between males. Because of the promiscuous mating system, simple censused-based measures of male reproductive success are poor indicators of true male success so paternity has been inferred using molecular genetic markers (Bancroft 1993; Pemberton *et al*. 1996; Smith 1996; Marshall *et al*. 1998).

(*c*) *Paternity analysis*

Paternity analysis has been carried out for 921 lambs born in the years 1986–94 and is described elsewhere (Pemberton *et al*. 1996; Smith 1996; Marshall *et al*. 1998). Lambs, mothers and candidate fathers (essentially, all tagged males known to be alive at any particular rut) were typed at up to 17 polymorphic loci comprising six allozyme and 11 microsatellite loci. One locus, OLADRB, a microsatellite within an expressed class II MHC gene, was used in both the paternity analysis and the mate-choice analysis described in this paper; the implications of this are explored below in §3. Paternity inference was made using a likelihood-based approach, supported by simulation, to discriminate fathers from among matching males (Marshall *et al*. 1998). In the following mate choice analysis, two data sets were analysed: (i) cases from among the 665 paternities assigned using a 'relaxed' criterion of 80% confidence in the paternity inference; and (ii) cases from among the 317 paternities assigned using a 'strict' criterion of 95% confidence.

(*d*) *MHC markers*

Mate choice on the basis of MHC type was analysed using five polymorphic microsatellite loci located within or adjacent to the MHC in recent ovine genetic maps (Crawford *et al*. 1995) as shown in figure 1. The markers OLADRB and OLADRBps are located within MHC class II expressed and non-expressed genes respectively (Blattman & Beh 1992; Schwaiger *et al*. 1993), OMHC1 is located within the MHC class I region (Groth & Wetherall 1994) and BM1815 and BM1818 (Bishop *et al*. 1994) were used as flanking marker controls.

Lambs sampled at birth in the 1988 to 1994 cohorts were typed at the five microsatellite loci using standard procedures in our lab which are described in Paterson (1998). Table 1 gives summary data for each locus. As reported more fully elsewhere (Paterson 1998), all five loci are in Hardy–Weinberg proportions (table 1) and loci within the MHC show high levels of linkage disequilibrium with each other but not with flanking markers (figure 1). Markers within the MHC should therefore be in linkage disequilibrium with any site(s) having a causal effect upon mate-choice and hence show associations with mate-choice. Flanking markers should show no such

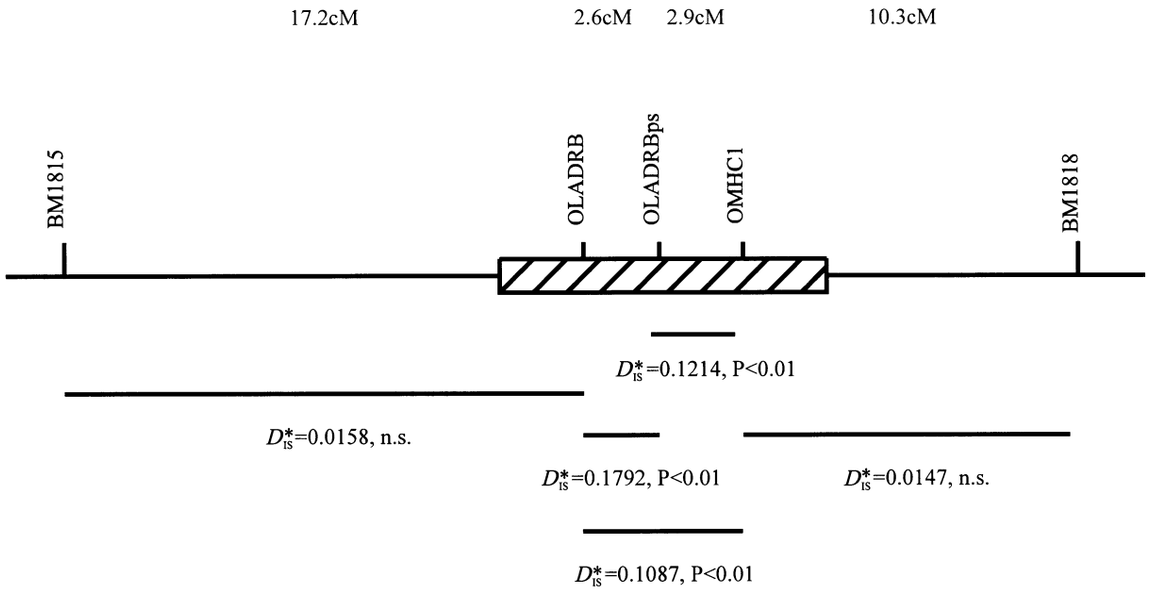

Figure 1. Part of sheep chromosome 20 showing the MHC (indicated by box) and the names and locations of the microsatellite markers used in this study. Genetic distances and nomenclature are taken from Crawford (1995). D^*_{IS}, the within cohort component of linkage disequilibrium adjusted for heterozygosity, and significance levels of linkage disequilibrium between neighbouring pairs of markers in the St Kilda Soay sheep population (from Paterson 1998) are also shown.

Table 1. *Summary population data for the five markers used in this study*

(Three microsatellites within the MHC (OLADRB, OLADRBps and OMHC1) and two microsatellites flanking the MHC (BM1815 and BM1818) were screened in new-born Soay lambs (1988–1994 cohorts). The numbers of individuals screened, the number of alleles found, and heterozygosity are shown for each locus. Hardy–Weinberg exact tests were calculated by the Markov-chain method for multiple alleles (Guo & Thompson 1992) and are described in more detail elsewhere (Paterson 1998). Sample sizes varied between loci due to time constraints on this project and due to the ease with which some loci amplified relative to others.)

marker	BM1815	OLADRB	OLADRBps	OMHC1	BM1818
number of animals screened	961	1209	887	1025	893
number of alleles	3	8	6	5	7
heterozygosity	0.506	0.796	0.788	0.581	0.655
Hardy–Weinberg exact test p value	0.16	0.97	0.27	0.17	0.38

associations. Complete correlation exists between one of the microsatellite markers, OLADRB and adjacent sequence polymorphism in the expressed class II gene *Ovar-DRB1* (Paterson 1998).

(*e*) *Mate choice analysis*

To investigate the possibility that matings may be influenced by the degree of similarity between MHC genotypes of parents, a novel likelihood-based method (Edwards 1972) was developed which, for each MHC marker, compared the genotypes of each lamb's mother and inferred father. The advantage of this technique over conventional tests for Hardy–Weinberg equilibrium is that first, assortment of MHC genotypes within matings are directly observed rather than inferred from population genotype frequencies, and second, specific questions concerning degree of MHC similarity between parents and the nature of any disassortative mating may be framed and directly incorporated into likelihood calculations.

Under random mating, the probability of parents sharing exactly 0, 1 or 2 alleles at a locus may be given by ϕ_0 ϕ_1 and ϕ_2 respectively:

$$\left.\begin{aligned}
\phi_0 &= \sum_{i=1}^{n}\sum_{j>i} 2p_ip_j(1-p_i-p_j)^2 + \sum_i p_i^2(1-p_i)^2,\\
\phi_1 &= \sum_{i=1}^{n}\sum_{j>i} 2p_ip_j(1-2p_ip_j-(1-p_i-p_j)^2)\\
&\quad + \sum_i 2p_i^3(1-p_i),\\
\phi_2 &= \sum_{i=1}^{n}\sum_{j>i} 4p_i^2p_j^2 + \sum_i p_i^4,
\end{aligned}\right\} \quad (1)$$

where p_i and p_j are frequencies of alleles i and j respectively for a locus with n alleles and $\phi_0 + \phi_1 + \phi_2 = 1$.

Table 2. *Analysis of mating preferences in relation to MHC markers*

(Paternities were assigned with either (*a*) 80% or (*b*) 95% confidence and the analysis of mating preferences was conducted with both data sets. The samples sizes shown vary between loci because not all individuals have been genotyped at each locus. The tables show the observed and, in brackets, expected numbers of sire–dam pairs sharing 0, 1 or 2 alleles at each of the five loci used in this study. The test statistic χ^2 distributed with 2 d.f. and p values indicate that St Kilda Soay sheep mating patterns are not associated with MHC genotype.)

marker	BM1815	OLADRB	OLADRBps	OMHC1	BM1818
(*a*)					
share 0 alleles (expected)	59 (47.25)	232 (244.76)	88 (88.99)	42 (48.02)	95 (94.65)
share 1 allele (expected)	171 (174.31)	253 (244.79)	107 (108.53)	146 (136.75)	196 (194.33)
share 2 alleles (expected)	96 (104.44)	33 (28.45)	18 (15.47)	40 (43.23)	47 (49.02)
total	326	518	213	228	338
$\chi^2_{v=2}$	3.486	1.637	0.424	1.647	0.100
p value	0.175	0.441	0.809	0.439	0.951
(*b*)					
share 0 alleles (expected)	23 (21.31)	129 (120.49)	39 (35.93)	19 (20.01)	46 (44.80)
share 1 allele (expected)	74 (78.60)	112 (120.51)	39 (43.82)	63 (56.98)	92 (91.99)
share 2 alleles (expected)	50 (47.09)	14 (14.01)	8 (6.25)	13 (18.01)	22 (23.20)
total	147	255	86	95	160
$\chi^2_{v=2}$	0.587	1.203	1.257	2.212	0.095
p value	0.746	0.548	0.533	0.331	0.953

The corresponding multinomial likelihood function, $L(\theta)$, may be given as:

$$L(\theta) = k[\phi_0]^{a_0}[\phi_1]^{a_1}[\phi_2]^{a_2}, \quad (2)$$

(Edwards 1972), where k represents an arbitrary constant and a_0, a_1 and a_2 the number of paternities where females and males share exactly 0, 1 and 2 alleles, respectively.

Non-random mating may be defined in terms of deviations from random expectations, $\phi'_0 = d_0\phi_0$, $\phi'_1 = d_1\phi_1$ and $\phi'_2 = d_2\phi_2$, such that $\phi'_0 + \phi'_1 + \phi'_2 = 1$, with the corresponding likelihood function:

$$L'(\theta) = k[\phi'_0]^{a_0}[\phi'_1]^{a_1}[\phi_2]^{a_2}, \quad (3)$$

which is maximized at

$$\phi'_0 = \frac{a_0}{a_0 + a_1 + a_2}, \ \phi'_1 = \frac{a_1}{a_0 + a_1 + a_2} \text{ and}$$
$$\phi'_2 = \frac{a_2}{a_0 + a_1 + a_2}.$$

The test statistic

$$2\ln L'(\theta) - 2\ln L(\theta), \quad (4)$$

is distributed as a χ^2 with 2 d.f.

The reliability of the statistic $2\ln L'(\theta)$ $2\ln L(\theta)$, under the assumptions of the model (see below), was tested by Monte-Carlo simulation of random mating using the observed allele frequencies and sample sizes at each of the five loci.

When applying this method, we treated each lamb as a data point. Since some males and females have multiple offspring, some parents make multiple appearances in the data set analysed here. The possible problems that may be introduced by this approach are discussed in the results section below.

(*f*) *Power of analysis*

We investigated the power of our analysis to detect deviations from random mating at the MHC following the method used by Hedrick & Black (1997). The probabilities of parents sharing 0, 1 or 2 alleles at each loci were weighted by 1, $1-s/2$ and $1-s$ respectively, and standardized to preserve a total probability of 1, where s represents selection against assortative mating. For each locus in both data sets used, the level of selection which would give rise to a 2 X loglikelihood ratio of 5.99 or more, indicating significance at the 5% level with 2 d.f., was determined. The levels of selection which would give rise to a 2 X loglikelihood ratio of 3.84, were also determined. This corresponds to significance at the 5% level with 1 d.f. under a simple model where selection against assortative mating is additive (i.e. that the level of selection against parents sharing 1 allele lies midway between the levels of selection against parents sharing either 0 or 2 alleles).

3. RESULTS

(*a*) *Mating preferences*

Table 2 shows the expected and observed number of matings in which parents share 0, 1 or 2 alleles at each of the five loci given the observed allele frequencies for data sets based upon (*a*) 80% and (*b*) 95% confidence in paternity inference. No significant deviations from random mating were observed at any of the five loci.

Monte-Carlo simulation of random mating using the observed allele frequencies and sample sizes at each of the five loci (1000 replicates in each case) conformed closely to the expected χ^2 distribution (results not shown) indicating that the results shown in table 2 are robust (under the assumptions of the model—see below).

There are three possible sources of deviation from the model assumptions. (i) Animals were allowed multiple appearances in the data set used, i.e. the data points might be non-independent. This effect, however, is expected to be slight since full sibs are rare (6%) and maternal and paternal half-sibships are generally small (mean maternal sibship size 1.92, mean paternal sibship size 1.84; Smith 1996). More importantly, non-independence of data would not be expected to lead to a systematic bias, although frequency of type I errors may increase as sample size decreases. (ii) Different

allele frequencies between males and females could be expected to distort the test statistic used here. However, no evidence of differences between male and female allele frequencies was observed, either on the basis of deviations from Hardy–Weinberg proportions or disequilibrium between loci independent of linkage (table 1; Paterson 1998). Different allele frequencies between males and females would in any case be expected to lead to an increase in the frequency of type I errors whereas no deviations from random mating were observed. (iii) One of the microsatellite markers within the MHC, OLADRB, was included in both paternity inference and mate-choice analysis. This was necessary since the efficiency of paternity analysis on the basis of molecular data is highly dependent on the number of loci used. Dropping the highly polymorphic locus OLADRB from the paternity analysis would have significantly reduced both the confidence with which paternities could be assigned and the size of the data sets available for analysis. Since OLADRB dissimilar sire–dam pairs are more likely to meet the likelihood criteria for paternity inference, if anything they should be over-represented in the data sets used in mate-choice analysis and lead to an excess of type I errors (false positives). In fact the data do not support this, sire–dam pairs show no significant deviations from the null model (random mating) at either MHC or flanking loci. In summary, if the analysis suffers unduly from type I errors, the confidence with which the null model of random mating can be accepted is therefore increased, further decreasing support for the hypothesis of mate-choice.

(*b*) *Power of analysis*

An important consideration in regard to the mate-choice test outlined in this paper is the power of such a test to detect deviations from random mating. This is especially true in this study where no significant deviations from random mating were observed. For the data set based upon 80% confidence in paterinty inference (table 2*a*), we would be able to detect deviations significant at the 5% level with selection in the range of 0.33 to 0.48 for the five loci. The data set based upon 95% confidence in paternity inference (table 2*b*) shows considerably less power to detect deviations from random mating, with significant deviations only detectable at selection values between 0.42 and 0.65. Hedrick (1992) estimated the level of selection detected by Potts *et al.* (1991) in their study of semi-natural mouse populations as 0.69. All of the tests made in this study would be capable of revealing such a level of selection.

Greater power, detecting more subtle effects can be achieved by making the simplifying assumption that the selective effects of mate-choice are additive. Selection levels in the range of 0.26 to 0.40 for the data set based upon 80% confidence of paternity inference, and 0.36 to 0.54 for the data set based upon 95% confidence of paternity inference can then be detected by our approach. Even with this approximation, however, no significant deviations from random mating were detected for any of the five loci in Soay sheep.

4. DISCUSSION

This study represents possibly the most extensive investigation of MHC-based mate choice in a free-living population to date. We have reported allele frequency distributions indicating balancing selection within the MHC at OLADRB and OLADRBps, two of the three markers located within the MHC (Paterson 1998), implying, in common with other studies, a selective force in the maintenance of MHC diversity and heterozygosity (Hedrick & Thomson 1983). In this paper we tested mate choice with respect to the MHC using a novel paternity-based approach. In the St Kilda Soay sheep population, neither heterozygote excess nor non-random association of parental alleles within matings was observed. In a separate analysis (Paterson *et al.* 1998), we have found associations between MHC variation, parasite resistance and survivorship in the study population. By itself, however, this does not preclude the possibility that mate choice also acts to increase MHC diversity. In fact, some researchers suggest that pathogen challenge may be a necessary prerequisite for MHC-based odour discrimination and mate choice (Wedekind 1994). However, no evidence was found for mate choice in the Soay population. We therefore believe mate-choice to be an unlikely mechanism for the maintenance of MHC polymorphism within this population.

The statistical method for detecting MHC-based mate-choice in this paper was developed specifically for the Soay population which, being isolated and genetically homogeneous, makes an excellent study population. Some of the problems associated with the statistical approach are outlined in §3. Additional problems may be encountered if this method is applied to other studies. First, subdivided populations, unless controlled for, would give higher numbers of MHC-similar matings and lower heterozygosity than expected. Second, in populations with high heterozygosity, statistical power may be greatly reduced, due to a low probability of parents sharing two alleles under random mating. Lastly, methods of inbreeding avoidance independent of MHC-type, e.g. avoiding full-sib matings, could also have a significant impact upon the degree of genetic similarity between parents leading to patterns which look similar to MHC-disassortative matings.

Although MHC-based mating preferences and selective abortion have been implicated in the maintenance of diversity at the MHC in studies of mice and humans (Hedrick 1994; Potts *et al.* 1991, 1994; Potts & Wakeland 1990; Wedekind *et al.* 1995, 1996), it is unclear how widespread the phenomenon of reproductive selection at the MHC is among vertebrates. Certainly there is no evidence that the MHC plays a significant role in the breeding system of the St Kildan Soay sheep population, and a recent study of Amerindian populations has also failed to find evidence for MHC-based mate choice (Hedrick & Black 1997). There are a number of possible explanations for the lack of MHC-based mating preferences detected in the Soay sheep. First, it is possible that the Soay sheep mating system does not involve any mate choice mechanism through which

such preferences could be expressed. Because of their investment in parental care, one would expect females to be the choosier sex (Trivers 1972), but in Soay sheep, matings appear to result wholly from competitive interactions between males and may offer little opportunity for female mate choice on the basis of odour profiles (Grubb 1974). Furthermore since, following a mating, males cannot be certain of paternity (Bancroft 1993; Stevenson & Bancroft 1995), selective mating of females may not be a viable male strategy. Finally, although we have not explicitly tested for it here, a mechanism involving selective abortion may be absent in this highly seasonal breeder since females have only a limited amount of time in which to conceive. Thus, one possibility is that evolution of mating systems involving mating preferences is a necessary requirement for the evolution of MHC-based patterns of mate choice.

Given evidence of parasite-driven selection at the MHC in both humans (Hill *et al*. 1991) and chickens (Briles *et al*. 1977), it may be the case that maintenance of MHC diversity by parasites is a more widespread and conserved phenomenon than mating preferences on the basis of MHC-derived odour profiles (reviewed in Potts & Wakeland (1990)). Despite a wide variety of parasites, the central role of the MHC in the immune response is conserved throughout vertebrates (Klein 1986; Trowsdale 1993). By contrast, vertebrate mating systems often show astounding variation even between closely related species (Harcourt *et al*. 1995; Temrin & Tullberg 1995). A single reproductive mechanism maintaining MHC diversity across all vertebrates therefore seems unlikely. Further studies like ours are required in a range of natural populations in order to determine first, the relative contributions of parasite-driven selection and reproductive selection in the maintenance of MHC polymorphism, and second, the types of mating systems which commonly support reproductive selection at the MHC.

We thank the numerous people who have helped collect samples and data on St Kilda, especially A. Robertson, A. MacColl and J. Pilkington; Scottish Natural Heritage and the National Trust for Scotland for permission to work there and the Royal Artillery Regiment (Hebrides) and the Royal Corps of Transport for logistical assistance. We also thank Nick Barton, Phil Hedrick and Ben Sheldon for valuable comments during the preparation of this manuscript. The study drew on paternity data previously generated by D. Bancroft, J. Smith and T. Marshall. The research was funded by BBSRC and NERC.

REFERENCES

Bancroft, D. R. 1993 Genetic variation in Soay sheep. PhD thesis, University of Cambridge, UK.

Bishop, M. D., Kappes, S. M., Keele, J. W., Stone, R. T., Sunden, S. L. F., Hawkins, G. A., Toldo, S. S., Fries, R., Grosz, M. D., Yoo, J. Y. & Beattie, C. W. 1994 A genetic-linkage map for cattle. *Genetics* **136**, 619–639.

Blattman, A. N. & Beh, K. J. 1992 Dinucleotide repeat polymorphism within the ovine major histocompatibility complex. *Anim. Genet.* **23**, 392.

Briles, W. E., Stone, H. A. & Cole, R. K. 1977 Marek's disease: effects of B histocompatibility alloalleles in resistant and susceptible chicken lines. *Science* **195**, 193–195.

Campbell, R. N. 1974 St Kilda and its sheep. In *Island survivors* (ed. P. A. Jewell, C. Milner & J. Morton Boyd), pp. 8–35. London: Athlone.

Clutton-Brock, T. H., Price, O. F., Albon, S. D. & Jewell, P. A. 1991 Persistent instability and population regulation in soay sheep. *J. Anim. Ecol.* **60**, 593–608.

Clutton-Brock, T. H., Price, O. F., Albon, S. D. & Jewell, P. A. 1992 Early development and population fluctuations in soay sheep. *J. Anim. Ecol.* **61**, 381–396.

Crawford, A. M., Dodds, K. G., Ede, A. J., Pierson, C. A., Montgomery, G. W., Garmonsway, H. G., Beattie, A. E., Davies, K., Maddox, J. F., Kappes, S. W., Stone, R. T., Nguyen, T. C., Penty, J. M., Lord, E. A., Broom, J. E., Buitkamp, J., Schwaiger, W., Epplen, J. T., Matthew, P., Matthews, M. E., Hulme, D. J., Beh, K. J., McGraw, R. A. & Beattie, C. W. 1995 An autosomal genetic-linkage map of the sheep genome. *Genetics* **140**, 703–724.

Ebert, D. & Hamilton, W. D. 1996 Sex against virulence: the coevolution of parasitic diseases. *Trends Ecol. Evol.* **11**, 79–82.

Edwards, A. W. F. 1972 *Likelihood*. Cambridge University Press.

Groth, D. M. & Wetherall, J. D. 1994 Dinucleotide repeat polymorphism within the ovine major histocompatibility complex class I region. *Anim. Genet.* **25**, 61.

Grubb, P. 1974 The rut and behaviour of Soay rams. In *Island survivors* (ed. P. A. Jewell, C. Milner & J. Morton Boyd), pp. 195–223. London: Athlone.

Guo, S. W. & Thompson, E. A. 1992 Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Harcourt, A. H., Purvis, A. & Lilles, L. 1995 Sperm competition mating system, not breeding season, affects testes size of primates. *Funct. Ecol.* **9**, 468–476.

Hedrick, P. W. 1992 Female choice and variation in the major histocompatibility complex. *Genetics* **132**, 575–581.

Hedrick, P. W. 1994 Evolutionary genetics of the major histocompatibility complex. *Am. Nat.* **143**, 945–964.

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

Hedrick, P. W. & Thomson, G. 1983 Evidence for balancing selection at HLA. *Genetics* **104**, 449–456.

Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. & Greenwood, B. M. 1991 Common West African HLA antigens are associated with protection from severe malaria. *Nature* **352**, 595–600.

Hughes, A. L. & Nei, M. 1988 Pattern of nucleotide substitution at major histocompatibility complex class-I loci reveals overdominant selection. *Nature* **335**, 167–170.

Hughes, A. L. & Nei, M. 1989 Nucleotide substitution at major histocompatibility complex class-II loci—evidence for overdominant selection. *Proc. Natn. Acad. Sci. USA* **86**, 958–962.

Keymer, A. E., Tarlton, A. B., Hiorns, R. W., Lawrence, C. E. & Pritchard, D. I. 1990 Immunogenetic correlates of susceptibility to infection with *Heligmosomoides polygyrus* in outbred mice. *Parasitology* **101**, 69–73.

Klein, J. 1986 *Natural history of the major histocompatibility complex*. New York: Wiley.

Klein, J., Satta, Y., Ohuigin, C. & Takahata, N. 1993 The molecular descent of the major histocompatibility complex. *A. Rev. Immunol.* **11**, 269–295.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Molec. Ecol.* (Submitted).

Paterson, S. 1998 Evidence for balancing selection at the major histocompatibility complex in a free-living ruminant. *J. Hered.* (Submitted.)

Paterson, S., Wilson, K. & Pemberton, J. M. 1998 Major histocompatibility complex (MHC) variation associated with juvenile survival and parasite resistance in a large

unmanged ungulate population (*Ovis aries* L.). *Proc. Natn. Acad. Sci., USA* (In the press.)

Pemberton, J. M., Smith, J. A., Coulson, T. N., Marshall, T. C., Slate, J., Paterson, S., Albon, S. D. & Clutton-Brock, T. H. 1996 The maintenance of genetic polymorphism in small island populations: large mammals in the Hebrides. *Phil. Trans. R. Soc. Lond.* B **351**, 745–752.

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

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1994 The role of infectious-disease, inbreeding and mating preferences in maintaining MHC genetic diversity—an experimental test. *Phil. Trans. R. Soc. Lond.* B **346**, 369378.

Potts, W. K. & Wakeland, E. K. 1990 Evolution of diversity at the major histocompatibility complex. *Trends Ecol. Evol.* **5**, 181–187.

Schwaiger, F. W., Buitkamp, J., Weyers, E. & Epplen, J. T. 1993 Typing of artiodactyl MHC-DRB genes with the help of intronic simple repeated sequences. *Molec. Ecol.* **2**, 55–59.

Schwaiger, F. W., Gostomski, D., Stear, M. J., Duncan, J. L., McKellar, Q. A., Epplen, J. T. & Buitkamp, J. 1995 An ovine major histocompatibility complex DRBl allele is associated with low fecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Int. J. Parasitol.* **25**, 815–822.

Singer, A. G., Beauchamp, G. K. & Yamazaki, K. 1997 Volatile signals of the major histocompatibility complex in male mouse urine. *Proc. Natn. Acad. Sci. USA* **94**, 2210–2214.

Singh, P. B., Brown, R. E. & Roser, B. 1987 MHC antigens in urine as olfactory recognition cues. *Nature* **327**, 161–164.

Smith, J. A. 1996 Polymorphism, parasites and fitness in Soay sheep. PhD thesis, University of Cambridge, UK.

Stevenson, I. R. & Bancroft, D. R. 1995 Fluctuating tradeoffs favour precocial maturity in male Soay sheep. *Proc. R. Soc. Lond.* B **262**, 267–275.

Temrin, H. & Tullberg, B. S. 1995 A phylogenetic analysis of the evolution of avian mating systems in relation to altricial and precocial young. *Behav. Ecol.* **6**, 296–307.

Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man* (ed. B. Campbell), pp. 136–179. London: Heinemann.

Trowsdale, J. 1993 Genomic structure and function in the MHC. *Trends Genet.* **9**, 117–122.

Wedekind, C. 1994 Mate choice and maternal selection for specific parasite resistances before, during and after fertilization. *Phil. Trans. R. Soc.* B **346**, 303–311.

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

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, J. 1995 MHC-dependent mate preferences in humans. *Proc. R. Soc. Lond.* B **260**, 245–249.

Yuhki, N. & O'Brien, S. J. 1997 Nature and origin of polymorphism in feline MHC class II *DRA* and *DRB* genes. *J. Immunol.* **158**, 2822–2833.

Received 19 August 1997; accepted 16 September 1997