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Evolution

Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*)

(genetic diversity/selection/helminth parasites/Red Queen)

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ABSTRACT **Antagonistic coevolution between hosts and parasites has been proposed as a mechanism maintaining genetic diversity in both host and parasite populations. In particular, the high levels of genetic diversity widely observed at the major histocompatibility complex (MHC) of vertebrate hosts are consistent with the hypothesis of parasite-driven balancing selection acting to maintain MHC genetic diversity. To date, however, empirical evidence in support of this hypothesis, especially from natural populations, has been lacking. A large unmanaged population of Soay sheep (*Ovis aries L.*) is used to investigate associations between MHC variation, juvenile survival, and parasite resistance. We show in an unmanaged, nonhuman population that allelic variation within the MHC is significantly associated with differences in both juvenile survival and resistance to intestinal nematodes. Certain MHC alleles are associated with low survivorship probabilities and high levels of parasitism or vice versa. We conclude that parasites are likely to play a major role in the maintenance of MHC diversity in this population.**

Maintenance of genetic diversity at the major histocompatibility complex (MHC) of vertebrates has become a paradigm for the manner in which genetic diversity may be maintained in natural populations (1). The MHC consists of a group of closely linked genes involved in antigen presentation to the vertebrate immune system (2) and is remarkable in that extremely high levels of heterozygosity are commonly observed at certain genes contained within the complex (1, 3).

Several lines of evidence indicate that the high allelic diversity observed at the MHC is maintained through some form of selective force. First, where genealogies of MHC allele sequences have been constructed from a number of species [for example, primates (4) and felines (5)] it is observed that the divergence of allelic MHC lineages predates the speciation event giving rise to separate taxa. This suggests the action of some form of balancing selection over long periods of evolutionary time. Second, comparisons of allelic sequences present within mice and human populations indicate that the rate of nonsynonymous (coding) substitution exceeds the rate of synonymous (noncoding) substitution at the antigen presenting site, thus favoring new MHC variants and increasing diversity (6–8). Finally, in human populations the large numbers of alleles present at MHC loci show a relatively even distribution, leading to higher levels of heterozygosity than may be explained under neutral theory (9) and suggesting the action of recent balancing selection. So far, however, most studies of MHC polymorphism have been confined to human or semicaptive mouse populations, and there is a paucity of population-based studies within natural populations of other vertebrates.

Despite strong evidence for balancing selection at the MHC, the mechanisms behind maintenance of MHC polymorphism are still unclear. Two broad categories of selective mechanisms have been proposed. Reproductive mechanisms, namely MHC-based mating preferences and selective abortion, have been documented in both mice and human populations and may show sufficiently strong effects to maintain MHC diversity (10–13). Parasite-driven mechanisms are a more obvious source of selection given the MHC's central role in the vertebrate immune system and will be the subject of this paper. Theory predicts that parasite-driven balancing selection may maintain MHC polymorphism in one of two ways; (*i*) Overdominance [heterozygote advantage (14)], whereby MHC heterozygosity increases the range of parasites recognized by the immune system, hence increasing the relative fitness of MHC heterozygotes compared with homozygotes. This is analogous to maintenance of genetic variation through random environmental fluctuations where the heterozygote "bet hedging" strategy is favored in a variable environment (15, 16). (*ii*) Negative frequency-dependent selection (17, 18), which considers host-parasite interactions as a dynamic process, with MHC alleles favored at low frequencies and rising in frequency, only to induce a corresponding shift in the genetic composition of the parasite population, which reduces the fitness of common host MHC alleles (for review see refs. 1 and 19).

We investigate the role of the MHC in survivorship and parasite resistance in a natural environment by using an unmanaged, individually monitored population of Soay sheep (*Ovis aries L.*) on the Scottish island of Hirta within the St. Kilda archipelago. In common with domestic flocks, this population is subject to high levels of intestinal parasitism, predominantly by strongyle nematodes. These parasites appear to play a major role in the life history of their hosts, with both experimental manipulations (20) and natural variation in parasite burden (21) showing a significant influence on juvenile survivorship.

In the Soay sheep population on St. Kilda, very even allele frequency distributions have been observed at microsatellite markers within the MHC—despite all loci showing Hardy–Weinberg equilibrium (55). These distributions are unlikely to have arisen under neutrality and suggests the action of rela-

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Abbreviations: MHC, major histocompatibility complex; FEC, fecal egg count.
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tively recent balancing selection. In addition, coding variation at *DRB*, a class II MHC gene, of Soay sheep indicates an excess of nonsynonymous compared with synonymous substitution, implying selection for MHC diversity over evolutionary time. No evidence for reproductive selection at the MHC has been found in this population (22). In this paper we investigate the role of MHC variation in juvenile survival and parasite resistance to examine selective effects of parasites within natural populations in the maintenance of MHC diversity.

MATERIALS AND METHODS

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 c. 5000 BC. It may have been introduced to St. Kilda as early as 2000 BC but since historic times has been restricted to the uninhabited island of Soay (23). In 1932, after the evacuation of the human population 2 years previously, 107 Soay sheep (20 rams, 44 ewes, 21 ewe lambs, and 22 castrated ram lambs) were introduced from Soay (99 hectares) to the larger island of Hirta (638 hectares) (23). Since this time, Soay sheep on Hirta have existed entirely unmanaged.

The population dynamics of Soay sheep on St. Kilda are characterized by periods of high over-winter mortality precipitated by food shortage. Over-winter mortality often occurs in severe crashes when up to 60% of the entire population may die from starvation with mortality highest in lambs and yearlings (24). After a rapid increase since their introduction onto Hirta in 1952, the total island population of Soay sheep has fluctuated between 600 and 1,800 animals. The population of sheep within the study area (approximately 200 hectares) represents around one-third of the total Hirta population, fluctuating in size between 200 and 600 animals, correlating closely with the fluctuation of the remaining island population (24, 25).

From 1985 to the present, at least 90% of lambs born within the study area were caught and individually tagged within a few days of birth. Blood samples and ear punches were taken from lambs at this time. In each subsequent August, as many animals as possible from all age classes have been caught and morphometric measurements taken (24, 25). In separate experiments by previous researchers, a small number of animals were treated with drugs. Antihelminthic was administered to relieve animals of helminth burden; these animals showed increased survivorship during the subsequent winter (20). Progesterone was administered to remove young rams from the November rut for a season; these animals also showed increased survivorship during the subsequent winter (26). Mortality is monitored by searching the study area for corpses during the spring and counting the animals throughout the year (24, 25).

Parasitological Work. Intestinal nematodes, particularly strongyle nematodes, are believed to be the major parasites of the Soay sheep of St. Kilda in terms of both prevalence and virulence (20, 27, 28). This hypothesis is in accord with similar studies on domestic flocks (29). These parasites cause significant damage to the abomasal mucosa and increase nutrient loss and protein deficiency in the gut (27, 30–32). Animals experimentally relieved of their intestinal nematode burden show a highly increased probability of over-winter survivorship (20). No evidence of viral pathogens have been found in this population (28). Fecal egg count (FEC) is used in this paper as a measure of helminth parasitism and host resistance. FEC is a preferred measure because it is (*i*) nondestructive and (*ii*) determined by numbers of parasites and by egg production of each parasite, factors which both may be influenced by the immune state of the host (33).

Fecal samples were taken from captured animals during each August since 1988. Numbers of strongyle eggs present in the feces (predominantly of the species *Teladorsagia circumcincta*, but also of the species *Teladorsagia davtiani*, *Ostertagia trifurcata*, *Trichostrongylus axei*, and *Trichostrongylus vitrinus*) were determined to the nearest 100 per gram by using a modification of the McMaster technique (27, 32).

MHC Markers. The MHC of sheep has yet to be fully investigated but it appears to have a similar structure to the HLA system of humans, with distinct class I and II regions each of which contain a number of expressed genes (2, 34). MHC variation was assayed by using five polymorphic microsatellite loci located within or adjacent to the MHC in recent ovine genetic maps (35) as shown in Fig. 1. The markers OLADRB and OLADRBps are located within MHC class II expressed and nonexpressed genes, respectively (36, 37). OMHC1 is

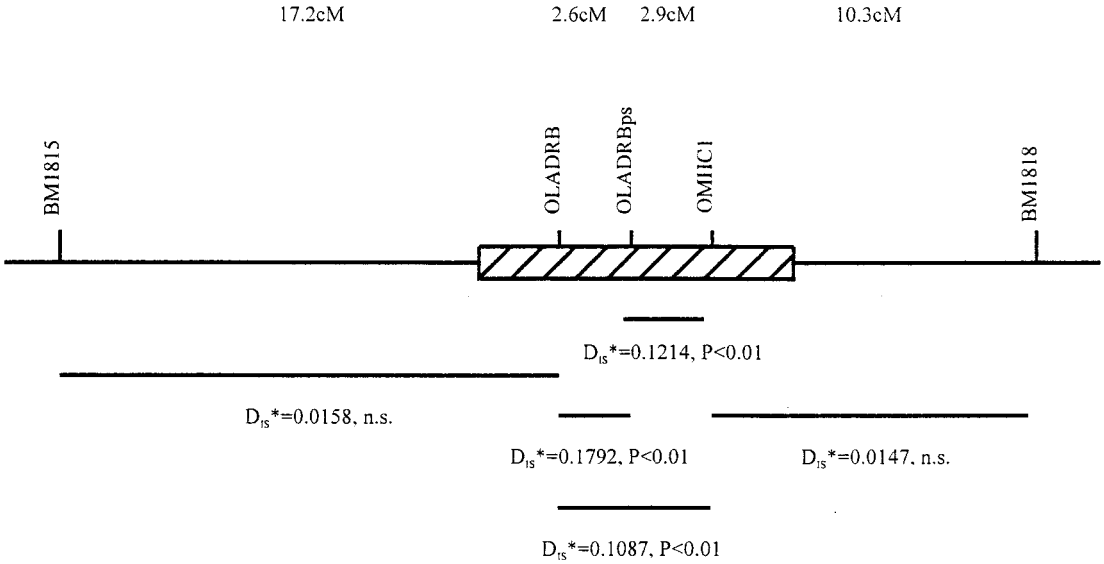

FIG. 1. Part of sheep chromosome 20 showing the MHC (indicated by box) and the names and locations of the microsatellite markers used in this study. Genetic distances and nomenclature are taken from Crawford (35). D_{IS}*, the within cohort component of linkage disequilibrium adjusted for heterozygosity, and significance levels of linkage disequilibrium between neighboring pairs of markers in the St. Kilda Soay sheep population (55) also are shown.

located within the MHC class I region (38), and BM1815 and BM1818 (39) were used as flanking marker controls.

Lambs sampled at birth in the 1988–1994 cohorts were typed at the five microsatellite loci by using standard procedures in our laboratory, which are described in ref. 55. Tables 1 and 2 gives summary data for each locus. As reported more fully elsewhere (55), all five loci are in Hardy–Weinberg equilibrium (Tables 1 and 2), and the three loci within the MHC show high levels of linkage disequilibrium with each other but not with flanking markers (Fig. 1). We use these markers to search for associations between MHC variation, survivorship, and parasite resistance. Markers within the MHC should be in linkage disequilibrium with any site(s) having a causal effect upon survivorship or parasite resistance and hence show associations with these traits. Flanking markers should show no such associations.

We note one marker in particular and place emphasis on it throughout this paper. The microsatellite OLADRB lies within an intron 30 bp downstream from the 3′ splice site of exon 2 in *Ovar-DRB1* (40), the major expressed class II *DRB* gene of sheep (41, 42). Class II genes are believed to play a major role in immune defense against macroparasites (43) and commonly show extensive polymorphism (3), the vast majority of this polymorphism being located in the antigen presenting site encoded by exon 2 (44). This pattern of polymorphism at *DRB* also is found in domestic sheep flocks (45, 46). High correlation is observed between microsatellite variation at OLADRB and adjacent, expressed sequence polymorphism, with all sequence variants distinguishable on the basis of microsatellite variation (55).

Statistical Treatment. Generalized linear models (47) were used in both analyses of juvenile survival and parasite resistance. Such models are commonly used to describe complex traits where many factors contribute toward a character (48). In this way we were able to control for many of the confounding variables present in the St. Kilda population that otherwise might obscure subtle genetic associations with the MHC. Minimal nongenetic models were constructed by using standard techniques (48) and the computer program SPLUS (Mathsoft, Cambridge, MA). A binomial error distribution was assumed for survivorship data, and a negative binomial error distribution was assumed for FEC data. In the case of lamb survival, only lambs that survived to 4 months of age were included in the analysis. This analysis excluded neonatal mortality, which may largely reflect maternal condition, and allowed measurements taken during August to be included in the model. Terms were deletion tested, with significance levels determined by comparing the resulting change in deviance against a χ^2 distribution with degrees of freedom equal to the number of terms (or levels of a factor) dropped (47).

After the construction of minimal nongenetic models, significance of genetic terms were calculated by deletion testing as before. Genetic terms were fitted either (*i*) under an additive model where, for a particular allele, the value of the heterozygote class lies midway between the two homozygote classes (i.e., given an allele A_i compared against all other alleles, denoted A_m, the genotypes A_mA_m, A_iA_m and A_iA_i have genotypic values 0, α_i and $2\alpha_i$ respectively), or (*ii*) under a symmetrical overdominance model where values of heterozygotes are compared against homozygotes (i.e., for all i, j≠i, the genotypes A_iA_i, A_iA_j, and A_jA_j have genotypic values 1-*d*, 1 and 1− *d*, respectively, where *d* denotes the change in the value of a trait because of overdominance). Alleles with a frequency of less than 2% were pooled with their closest length variant at that locus to prevent comparisons being made on small sample sizes. In all models, all comparisons made involved a minimum cell size of at least 30 data points (48).

Table 2. Frequency data for OLADRB

		Homozygotes		Heterozygotes	
Allele	Frequency	Observed	Expected	Observed	Expected
205	0.211	60	53.39	386	399.23
213	0.113	12	15.31	247	240.37
257	0.236	76	66.8	414	432.41
263	0.133	28	21.22	263	276.56
267	0.158	37	29.79	304	318.42
269	0.001	0	0.00	3	3.00
276	0.141	31	23.68	275	289.64
287	0.008	0	0.07	18	17.86

Frequency of each allele at OLADRB shown with observed and expected numbers of homozygotes and heterozygotes. Expected frequencies of homozygotes and heterozygotes are calculated from Hardy–Weinberg proportions as p_i^2 and $2p_i(1 - p_i)$, respectively, where p_i is the frequency of each allele.

RESULTS

Juvenile Survival. Mortality in Soay sheep is highest in lambs and yearlings (24), hence survivorship within these age classes is likely to be a strong predictor of fitness. Separate generalized linear models were constructed for lamb and yearling survival to avoid pseudo-replication. Mean lamb survivorship was found to be 0.471, and mean yearling survivorship was found to be 0.636. Significant nongenetic terms are shown in Table 3. In common with previous studies (21, 24), cohort, sex, and August weight were found to be important factors associated with juvenile survivorship.

Genetic terms were tested at each of the five loci. Given the alternate hypotheses of frequency-dependent selection or heterozygote advantage proposed to maintain MHC diversity, genetic terms were fitted under either an additive model or a symmetrical overdominance model. As shown in Tables 4 and 5, associations were observed at all three of the MHC loci when genetic terms were fitted simultaneously under an additive model but, with the exception of one marginally significant association (Table 5), not under an overdominance model. No significant associations were observed at either of the flanking marker controls (with one marginally significant exception, Table 5). Significant associations were observed in lambs as weight interactions and in yearlings as simple terms. Genetic terms also were fitted under a dominant model (i.e., given an allele A_i compared against all other alleles, denoted A_m, the genotypes A_mA_m, A_iA_m, and A_iA_i have genotypic values 0, α_i and α_i, respectively); for both lambs and yearlings the results

Table 1. Population data for all five markers

Marker	BM1815	OLADRB	OLADRBps	OMHC1	BM1818
No. of animals screened	961	1,209	887	1,025	893
No. 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

Three microsatellites within the MHC (OLADRB, OLADRBps, and OMHC1) and two microsatellites flanking the MHC (BM1815 and BM1818) were screened in newborn Soay lambs (1985–1994 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 (54).

Table 3. Nongenetic terms significantly associated with juvenile survivorship

Terms	Data type	d.f.	Δdeviance	*P* value
Lamb survival (Null model $n = 619$, deviance = 857.64)				
Cohort*	Factor	9	305.83	<0.001
Weight	Continuous	1	7.50	0.006
Sex	Factor	1	3.80	0.051
Treatment†	Factor	2	7.69	0.021
Weight × cohort	Interaction	9	45.04	<0.001
Sex × cohort	Interaction	9	32.54	<0.001
Sex × weight	Interaction	1	8.17	0.004
Residual	—	587	477.68	—
Yearling survival (Null model $n = 370$, deviance = 489.72)				
Cohort*	Factor	9	127.47	<0.001
Weight	Continuous	1	0.87	0.350
Treatment†	Factor	2	6.29	0.043
Sex	Factor	1	15.64	<0.001
Weight × cohort	Interaction	9	28.39	0.001
Residual	—	348	294.53	—

Nongenetic terms fitted to lamb survivorship and yearling survivorship in generalized linear model assuming a logistic error distribution. Significance of terms determined by deletion testing. Change in deviance for lower order terms were calculated by deletion from models not containing higher order terms.
*1985–1994 cohorts.
†Antihelminthic treated vs. progesterone treated vs. untreated.

from dominant models followed closely those produced under an additive model (results not shown).

The biological significance of the MHC × weight interactions observed with lamb survival remains unclear. MHC × weight interactions may occur in this model either (*i*) because of an effect of the MHC on weight (possibly mediated by parasites or by energetic costs associated with immune function) and hence on survivorship or (*ii*) through a mechanism whereby effects of the MHC on survivorship are dependent on weight (48).

The strongest associations with survivorship were observed with the locus OLADRB and are shown in Table 6. In lambs, allele 257 showed a significant weight interaction ($P < 0.01$), which appeared to decrease survivorship (estimated survivorship difference = −0.012), with the effect most pronounced in lambs below average weight; these underweight animals are most vulnerable to over-winter starvation (Table 3, refs. 21 and 24). In yearlings, allele 205 was associated with decreased survivorship (estimated survivorship difference = −0.089, $P < 0.01$), and allele 263 was associated with increased survivorship (estimated survivorship difference = +0.076, $P < 0.05$). Associations with alleles at OLADRBps and OMHC1 also were observed consistent with the linkage disequilibrium between alleles at these loci and alleles at OLADRB (not shown). Survival differences for alleles were estimated by comparing the mean fitted survival probability of animals carrying zero or one copy of the allele (numbers of animals homozygous for the allele generally were too small for robust analysis). This approach makes no assumptions as to the cause of any allele × weight interactions found and only looks at the overall difference between animals with zero or one copy of the allele.

Parasite Resistance. Separate generalized linear models were constructed for lamb and yearling FEC. A negative binomial error distribution was assumed (49, 50). Significant nongenetic terms are shown in Table 7. Mean fitted lamb FEC was found to be 409 eggs/g; mean fitted yearling FEC was found to be 248 eggs/g. Genetic terms were tested as before. Strongest associations were observed at OLADRB under an additive model (shown in Table 8). For lambs, the 257 allele under an additive model showed a highly significant association ($P < 0.01$), which acted to increase FEC by an estimated 104 eggs/g (for animals carrying one copy versus animals carrying 0 copies of the 257 allele). In yearlings, allele 263 acted to decrease FEC (−76 eggs/g, $P < 0.05$) and allele 267 acted to increase FEC (+96 eggs/g, $P < 0.05$). No associations were observed under an overdominance model for either lambs or yearlings. Genetic terms also were fitted under a dominant model and followed closely the results produced from the additive model (results not shown).

Sire Effects. A possible criticism of the methodology used here is that the analysis may be influenced by family structure. In particular, if rams sire many offspring, any associations found may be caused by genome-wide, rather than MHC-specific, sire effects. This effect would not be expected to lead to any systematic bias but would lead to an increase in the frequency of type I errors. This effect, however, is expected to be slight because sibships are generally small (mean maternal sibship size 1.92, mean paternal sibship size 1.84; ref. 51). More importantly, the use of flanking markers in this study limits any associations found to the MHC region rather than to genome-wide sire effects.

DISCUSSION

We previously have reported evidence of balancing selection on both allele frequency distributions and patterns of nucleotide substitution at the MHC (55). In this paper we present direct evidence of selection at the MHC. We examined MHC associations with survivorship in both lambs and yearlings—age classes in which highest mortality is observed (24). Associations with juvenile survival were observed at each of the three MHC loci when fitted under an additive model but not at either of the flanking markers, limiting associations to the MHC region. The general picture that emerges of selection at the MHC is that presence of individual alleles rather than heterozygosity is the critical factor determining mortality in lambs and yearlings with respect to MHC type.

At OLADRB, the locus at which the strongest associations with juvenile survivorship are observed, alleles significantly associated with strongyle parasite resistance in lambs and yearlings show consistent associations in juvenile survival. In

Table 4. Genetic associations with lamb survivorship

	Additive model						Symmetrical overdominance model					
	Locus term		Locus × weight interaction				Heterozygosity term		Heterozygosity × weight interaction			
Locus	Δdeviance	*P* value	Δdeviance	*P* value	*n*	d.f.	Δdeviance	*P* value	Δdeviance	*P* value	*n*	d.f.
BM1815	0.71	0.701	0.68	0.710	414	2	1.8	0.180	1.00	0.318	414	1
OLADRB	3.65	0.601	**12.93**	**0.024**	509	5	0.00	0.966	1.69	0.193	509	1
OLADRBps	4.07	0.539	**11.58**	**0.041**	398	5	0.52	0.471	0.03	0.870	398	1
OMHC1	3.00	0.557	**10.35**	**0.035**	484	4	0.63	0.428	0.78	0.378	484	1
BM1818	0.75	0.945	3.99	0.407	376	4	0.21	0.650	2.11	0.146	376	1

Genetic terms were fitted to a minimal model of lamb survivorship under an additive model and a symmetrical overdominance model (see text). Markers within the MHC are shown in italics. Significant values are shown in bold.

Table 5. Genetic associations with yearling survivorship

	Additive model						Symmetrical overdominance model					
	Locus term		Locus × weight interaction				Heterozygosity term		Heterozygosity × weight interaction			
Locus	Δdeviance	*P* value	Δdeviance	*P* value	*n*	d.f.	Δdeviance	*P* value	Δdeviance	*P* value	*n*	d.f.
BM1815	4.08	0.130	2.37	0.305	237	2	**3.88**	**0.049**	0.02	0.893	237	1
OLADRB	**13.94**	**0.016**	4.5	0.480	291	5	1.33	0.249	1.88	0.170	291	1
OLADRBps	**13.04**	**0.023**	0.7	0.983	210	5	2.86	0.091	2.30	0.129	210	1
OMHC1	**9.8**	**0.044**	3.76	0.439	242	4	**4.06**	**0.044**	3.78	0.052	242	1
BM1818	4.24	0.374	3.17	0.530	226	4	0.36	0.549	1.43	0.232	226	1

Genetic terms were fitted to a minimal model of yearling survivorship under an additive model and a symmetrical overdominance model (see text). Markers within the MHC are shown in italics. Significant values are shown in bold.

particular, the OLADRB 257 allele is significantly associated with both decreased parasite resistance and decreased survival in lambs; conversely, the OLADRB 263 allele is associated with both increased parasite resistance and increased survival in yearlings. No significant associations with parasite resistance are seen for the 205 allele that is associated with yearling survival. The mechanism behind these survivorship differences for this allele is unclear—possibly the 205 allele is associated with some other fitness trait as yet unidentified.

The consistency between alleles associated with survivorship and resistance to strongyle parasites suggests that particular MHC types confer either increased or decreased levels of parasite resistance that are translated into survivorship differences. Given this, it is reasonable to suppose that parasites play a major role in the maintenance of MHC polymorphism in the Soay population. These results are similar to those found by Hill *et al.* (52) in a large study of human malaria in West Africa. There it was found that certain MHC alleles were associated with protection from severe cerebral malaria in children—a condition that untreated would be likely to cause death in these individuals. Our results, however, show parasite-associated selection at the MHC in an unmanaged, nonhuman population.

It is tempting to draw conclusions with respect to the hypotheses of frequency-dependent selection and overdominance proposed to maintain MHC diversity. It was observed that particular MHC alleles, rather than heterozygosity, were associated with survivorship differences in both lambs and yearlings. Why then do favored alleles not rise to fixation in the population? In this respect, it is interesting to note that the most common alleles at OLADRB, 205 and 257, were associated with decreased survivorship, whereas the rarer 263 allele was associated with increased survivorship—as might be predicted under negative frequency-dependent selection. However, associations were not found to be consistent between lambs and yearlings. It may be the case that different MHC alleles exhibit different associations at different stages during the Soay sheep's life, possibly reflecting the complex interplay between helminth parasites and the vertebrate immune system (53). This could lead to heterozygotes showing highest overall fitness.

Table 6. Association between alleles at OLADRB and survivorship

OLADRB allele	Coefficient lamb survival (±SE)	Coefficient yearling survival (±SE)
205	0.026 (±0.055)	−0.898 (±0.310)**
213	0.005 (±0.050)	−0.004 (±0.438)
257	−0.181 (±0.061)**	0.311 (±0.293)
263	0.085 (±0.053)	0.726 (±0.359)*
267	0.091 (±0.080)	−0.423 (±0.400)
276	−0.029 (±0.057)	0.384 (±0.382)

Alleles at the class II MHC marker OLADRB were fitted under an additive model to lamb and yearling survivorship. Coefficients are shown for weight interactions in lamb survival and main effects in yearling survival. These coefficients are derived from a generalized linear model assuming a binomial error distribution (see text). *, $P < 0.05$; **, $P < 0.01$.

Despite this caveat, there is no evidence that alleles disadvantaged in lamb survivorship are advantageous in yearling survivorship or vice versa, but the possibility remains that MHC alleles may show associations with other, uncharacterized fitness traits, such as survivorship in older age classes or fecundity. Similarly, although the parasites considered here, strongyle nematodes, are thought to be the major parasites of both Soay and domestic flocks (29, 32), it is possible that other, perhaps uncharacterized parasite species exert strong selective effects on Soay sheep. Other MHC types therefore may confer protection against parasite species not considered in this study. The acid test of frequency-dependent selection would be to follow allele frequencies and selection coefficients through time and observe cycling of allele frequencies around a central mean. Unfortunately, the pace of change in allele frequencies and selection coefficients is likely to be limited by the average life span of Soay sheep (around 3 years), which is not much shorter than the number of cohorts available for this study (1985–1994).

Parasites represent a major force in the natural environment and maintenance of MHC diversity by parasite selection may prove to be a widespread phenomenon in vertebrates. Large-scale studies of this kind will be essential to explore the

Table 7. Nongenetic terms significantly associated with parasite resistance

Terms	Data type	d.f.	Δdeviance	*P* value
August lamb FEC (Null model $n = 386$, deviance = 582.62)				
Cohort*	Factor	6	73.38	<0.001
Sex	Factor	1	17.66	<0.001
Treatment†	Factor	1	0.57	0.449
Weight	Continuous	1	15.63	<0.001
Weight × sex	Interaction	1	8.81	0.003
Cohort × sex	Interaction	6	15.17	0.019
Treatment × weight	Interaction	1	6.31	0.012
Residual	—	369	432.30	—
August yearling FEC (Null model $n = 207$, deviance = 293.54)				
Cohort*	Factor	6	12.13	0.059
Sex	Factor	1	35.84	<0.001
Treatment‡	Factor	2	9.46	0.009
Weight	Continuous	1	6.87	0.009
Residual	—	197	229.55	—

Nongenetic terms fitted to lamb FEC and yearling FEC in a generalized linear models assuming a negative binomial error distribution. Significance of terms was determined by deletion testing. Change in deviance for lower order terms are calculated by deletion from models not containing higher order terms.

*1988–1994 cohorts.

†Progesterone treated vs. untreated.

‡Antihelminthic treated vs. progesterone treated vs. untreated.

Table 8. Association between alleles at OLADRB and parasite resistance

OLADRB allele	Coefficient lamb FEC (±SE)	Coefficient yearling FEC (±SE)
205	−0.119 (±0.089)	0.115 (±0.117)
213	−0.070 (±0.106)	−0.121 (±0.177)
257	0.252 (±0.077)**	−0.159 (±0.117)
263	−0.036 (±0.099)	−0.330 (±0.145)*
267	−0.009 (±0.091)	0.340 (±0.139)*
276	−0.179 (±0.101)	0.134 (±0.150)

Alleles at the class II MHC marker OLADRB were fitted under an additive model to lamb and yearling FEC. Coefficients are shown for main effects in lamb and yearling FEC. These coefficients are derived from a generalized linear model assuming a negative binomial error distribution (see text). *, $P < 0.05$; **, $P < 0.01$.

potential of parasites in the maintenance of genetic diversity at the MHC and other loci.

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1. Hedrick, P. W. (1994) *Am. Nat.* **143,** 945–964.
2. Trowsdale, J. (1993) *Trends Genet.* **9,** 117–122.
3. Klein, J., Takahata, N. & Ayala, F. J. (1993) *Sci. Amer.* **269,** 78–83.
4. Klein, J., Satta, Y., Ohuigin, C. & Takahata, N. (1993) *Annu. Rev. Immunol.* **11,** 269–295.
5. Yuhki, N. & O'Brien, S. J. (1997) *J. Immunol.* **158,** 2822–2833.
6. Hughes, A. L. & Nei, M. (1988) *Nature (London)* **335,** 167–170.
7. Hughes, A. L. & Nei, M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 958–962.
8. Hughes, A. L., Hughes, M. K., Howell, C. Y. & Nei, M. (1994) *Philos. Trans. R. Soc. London B* **345,** 359–367.
9. Hedrick, P. W. & Thomson, G. (1983) *Genetics* **104,** 449–456.
10. Wedekind, C., Seebeck, T., Bettens, F. & Paepke, J. (1995) *Proc. R. Soc. London B* **260,** 245–249.
11. Wedekind, C., Chapuisat, M., Macas, E. & Rulicke, T. (1996) *Heredity* **77,** 400–409.
12. Potts, W. K., Manning, C. J. & Wakeland, E. K. (1994) *Philos. Trans. R. Soc. London B* **346,** 369–378.
13. Potts, W. K., Manning, C. J. & Wakeland, E. K. (1991) *Nature (London)* **352,** 619–621.
14. Doherty, P. C. & Zinkernagel, R. M. (1975) *Nature (London)* **256,** 50–52.
15. Hedrick, P. W. (1986) *Annu. Rev. Ecol. Syst.* **17,** 535–566.
16. Ellner, S. & Haiston, N. G. (1994) *Am. Nat.* **143,** 403–417.
17. Hamilton, W. D. (1980) *Oikos* **35,** 282–290.
18. Bodmer, W. (1972) *Nature (London)* **237,** 139–145.
19. Potts, W. K. & Wakeland, E. K. (1993) *Trends Genet.* **9,** 408–412.
20. Gulland, F. M. D., Albon, S. D., Pemberton, J. M., Moorcroft, P. R. & Clutton-Brock, T. H. (1993) *Proc. R. Soc. London B* **254,** 7–13.
21. Illius, A. W., Albon, S. D., Pemberton, J. M., Gordon, I. J. & Clutton-Brock, T. H. (1995) *J. Anim. Ecol.* **64,** 481–492.
22. Paterson, S. & Pemberton, J. (1998) *Proc. R. Soc. London B* **264,** 1813–1819.
23. Campbell, R. N. (1974) in *Island Survivors*, eds. Jewell, P. A., Milner, C. & Morton Boyd, J. (Athlone, London), pp. 8–35.
24. Clutton-Brock, T. H., Price, O. F., Albon, S. D. & Jewell, P. A. (1992) *J. Anim. Ecol.* **61,** 381–396.
25. Clutton-Brock, T. H., Price, O. F., Albon, S. D. & Jewell, P. A. (1991) *J. Anim. Ecol.* **60,** 593–608.
26. Stevenson, I. R. & Bancroft, D. R. (1995) *Proc. R. Soc. London B.* **262,** 267–275.
27. Gulland, F. M. D. (1992) *Parasitology* **105,** 493–503.
28. Cheyne, I. A., Foster, W. M. & Spence, J. B. (1974) in *Island Survivors*, eds. Jewell, P. A., Milner, C. & Morton Boyd, J. (Athlone, London), pp. 338–359.
29. Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. & Jennings, F. W. (1987) *Veterinary Parasitology* (Longman, Avon).
30. Abbott, E. M., Parkins, J. J. & Holmes, P. H. (1985) *Res. Vet. Sci.* **38,** 6–13.
31. Coop, R. L., Huntley, J. F. & Smith, W. D. (1995) *Res. Vet. Sci.* **59,** 24–29.
32. Gulland, F. M. D. & Fox, M. (1992) *Parasitology* **105,** 481–492.
33. Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E. & Murray, M. (1995) *Parasite Immunol.* **17,** 643–652.
34. Trowsdale, J. (1995) *Immunogenetics* **41,** 1–17.
35. 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., *et al.* (1995) *Genetics* **140,** 703–724.
36. Schwaiger, F. W., Buitkamp, J., Weyers, E. & Epplen, J. T. (1993) *Mol. Ecol.* **2,** 55–59.
37. Blattman, A. N. & Beh, K. J. (1992) *Anim. Genet.* **23,** 392.
38. Groth, D. M. & Wetherall, J. D. (1994) *Anim. Genet.* **25,** 61.
39. 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) *Genetics* **136,** 619–639.
40. Schwaiger, F. W. & Epplen, J. T. (1995) *Immunol. Rev.* **143,** 199–224.
41. Ballingall, K. T., Wright, H., Redmond, J., Dutia, B. M., Hopkins, J., Lang, J., Deverson, E. V., Howard, J. C., Puri, N. & Haig, D. (1992) *Anim. Genet.* **23,** 347–359.
42. Dutia, B. M., McConnell, I., Ballingall, K. T., Keating, P. & Hopkins, J. (1994) *Anim. Genet.* **25,** 235–241.
43. Weir, D. W. & Stewart, J. (1993) *Immunology* (Churchill Livingston, Edinburgh).
44. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332,** 845–850.
45. Schwaiger, F. W., Weyers, E., Buitkamp, J., Ede, A. J., Crawford, A. & Epplen, J. T. (1994) *Mol. Biol. Evol.* **11,** 239–249.
46. Schwaiger, F. W., Weyers, E., Epplen, C., Brun, J., Ruff, G., Crawford, A. & Epplen, J. T. (1993) *J. Mol. Evol.* **37,** 260–272.
47. McCullagh, P. & Nelder, J. A. (1989) *Generalized Linear Models* (Chapman & Hall, London).
48. Crawley, M. J. (1993) *GLIM for Ecologists* (Blackwell, Oxford).
49. Wilson, K., Grenfell, B. T. & Shaw, D. J. (1996) *Funct. Ecol.* **10,** 592–601.
50. Wilson, K. & Grenfell, B. T. (1997) *Parasitol. Today* **13,** 33–38.
51. Smith, J. A. (1996) Ph.D. thesis (University of Cambridge).
52. 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) *Nature (London)* **352,** 595–600.
53. Maizels, R. M., Bundy, D. A. P., Selkirk, M. E., Smith, D. F. & Anderson, R. M. (1993) *Nature (London)* **365,** 797–805.
54. Guo, S. W. & Thompson, E. A. (1992) *Biometrics* **48,** 361–372.
55. Paterson, S. (1998) *J. Hered*., in press.