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A long-term genetic survey of an ungulate population reveals balancing selection acting on MHC through spatial and temporal fluctuations in selection

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We explored a 13-year genetic survey of the major histocompatibility complex (MHC) and neutral loci of the Soay sheep population of St Kilda to test the existence and causes of balancing selection at the MHC. The sheep population experiences demographic fluctuations, partly driven by the nematode *Teladorsagia circumcincta*. The spatial differentiation detected at the MHC was comparable to that at neutral loci between 1988 and 1996, but significantly lower between 1996 and 2000. The rate of temporal genetic differentiation was higher at the MHC, but within the Eastern heft only. These comparisons of spatial and temporal divergence at MHC and non-MHC loci provide strong evidence of balancing selection at the MHC, acting through spatial and temporal heterogeneity in selection pressure. This heterogeneity could be due to fluctuations in the selection imposed by parasites, either directly, because the prevalence in *T. circumcincta* varies in space and time, or indirectly, because the fitness costs of infection may vary with resource availability.
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Introduction

The major histocompatibility complex (MHC) is a central component of the vertebrate immune system. The genes encode cell-surface glycoproteins that are responsible for the recognition and presentation of antigens to T cells (Klein, 1986). Certain of these genes are among the most polymorphic regions of the vertebrate genomes (Klein, 1986). This pattern could be explained by some form of balancing selection (Hedrick and Thompson, 1983). Pathogens and parasites have been viewed as the main obvious agents driving this balancing selection (see for reviews Hughes and Yeager, 1998; Meyer and Thomson, 2001; Bernatchez and Landry, 2003). Three nonexclusive mechanisms have been proposed:

(i) *Negative frequency-dependent selection*: MHC genotypes with a rare allele are supposed to have a strong selective advantage as few pathogens have been exposed and adapted to it. Conversely, once alleles become frequent, pathogens adapt to them and are selected for, which decreases the fitness of MHC genotypes bearing these alleles (Meyer and Thomson, 2001). In support of this model, many associations between MHC alleles and resistance to parasitism have been found in natural populations (Hill *et al*, 1991; Paterson *et al*, 1998).

(ii) *Symmetrical overdominance*: Individuals that are heterozygous at particular MHC loci might be more resistant to particular infectious diseases than the corresponding homozygotes, because heterozygotes could present a wider repertoire of antigens (Doherty and Zinkernagel, 1975). Although this heterozygote selective advantage has been observed in natural populations (eg in humans; Carrington *et al*, 1999) and in animals (Penn *et al*, 2002), additional frequency dependence is also detected in these studies. Moreover theoretical models have recently shown that frequency dependence is required to explain the levels of polymorphism observed at MHC loci even under the hypothesis of overdominance (De Boer *et al*, 2004).

(iii) *Fluctuation in selection pressure*: The intensity and target selection may constantly change in time or space with the frequency of pathogens (Hedrick and Thompson, 1987), thus maintaining polymorphism at the level of a metapopulation. Fluctuating selection has been theoretically explored and validated (Hedrick, 2002) and several empirical studies support this idea of heterogeneity in selection pressure (see for a review Bernatchez and Landry, 2003). Variation in the presence or density of pathogens could account for this heterogeneity (Landry and Bernatchez, 2001; Miller *et al*, 2001; Hedrick, 2002).

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The free-living Soay sheep population on the island of Hirta (St Kilda archipelago, Scotland) is a good model in which to study the relative roles of a number of factors in shaping MHC genetic variation: changes over space and time in the environment and hence selection, population structure and demography. Historically, the population was restricted to the uninhabited island of Soay (St Kilda archipelago) where they survived unmanaged for between 1000 and 2000 years (Boyd and Jewell, 1974). In 1932, 2 years after the evacuation of the human population, 107 Soay sheep were introduced to the larger island of Hirta (638 ha). Since then, the population has remained unmanaged, and from 1985 until nowadays, it has been the subject of long-term individual demographic, parasitic and genetic surveys, providing an incomparable dataset to analyse the microevolutionary processes driving the evolution of a population. The population size of Soay sheep in Hirta fluctuates between 600 and 2000 individuals (see Figure 1), showing periods of rapid increase to high density, followed by periodic over winter 'crashes' when up to 60% of the population can die in one winter (Grenfell *et al*, 1992). The proximate cause of this mortality is starvation, but this malnutrition is exacerbated by protein and nutrient deficiency caused by the strongyle parasites, especially *Teladorsagia circumcincta* (Gulland, 1992). Within the study area, there is a spatial variation of faecal egg counts (FEC) of gastrointestinal nematodes, reflecting the parasite intensity (Wilson *et al*, 2003). It is partly maintained by variation in the density of sheep in space. It may also be influenced by variation in local topography and microclimate, and by variation in the quality of the sheep themselves (eg genotype, body condition – see Wilson *et al*, 2003). A recent study even reveals a significant genetic structure within the three spatial subunits (called hefts) in the Village Bay population (Coltman *et al*, 2003). These observations thus lead to the question of coevolution between the sheep and the strongyle populations. Recent studies have shown that there is a genetic component to resistance to this parasite. First, this trait, as measured by FEC, is heritable (Coltman *et al*, 1999; Smith *et al*, 1999). Second, there are locus-specific associations with parasite resistance (see, for example, Gulland *et al*, 1993; Coltman *et al*, 2001). In particular, a study conducted on five successive cohorts revealed that OLADRB3 alleles are associated with differences in juvenile survival and resistance to *T. circumcincta* (Paterson *et al*, 1998). Two other lines of evidence, the observation of the even allele frequency distribution and the excess of nonsynonymous compared to synonymous substitutions at MHC loci (Paterson, 1998), suggest that balancing selection could be in operation at this locus.

The aim of this study was to explore the large Soay sheep genetic data set provided by a 13-year survey of neutral and MHC loci to test for the hypothesis of balancing selection driven by fluctuating selection at the MHC. This is a rare opportunity to test the hypothesis of temporal variation, which still remains neglected (but see Seddon and Ellegren, 2004; Westerdahl *et al*, 2004). Lower levels of genetic differentiation in space (between the hefts) or in time (through years) at MHC than at neutral loci would suggest balancing selection without local selective constraints (Schierup *et al*, 2000; Muirhead, 2001). On the other hand, higher levels of spatial or temporal genetic differentiation observed at the MHC than at the neutral loci would be likely to result from environmental heterogeneity in the direction or the intensity of selection pressure (Bernatchez and Landry, 2003).

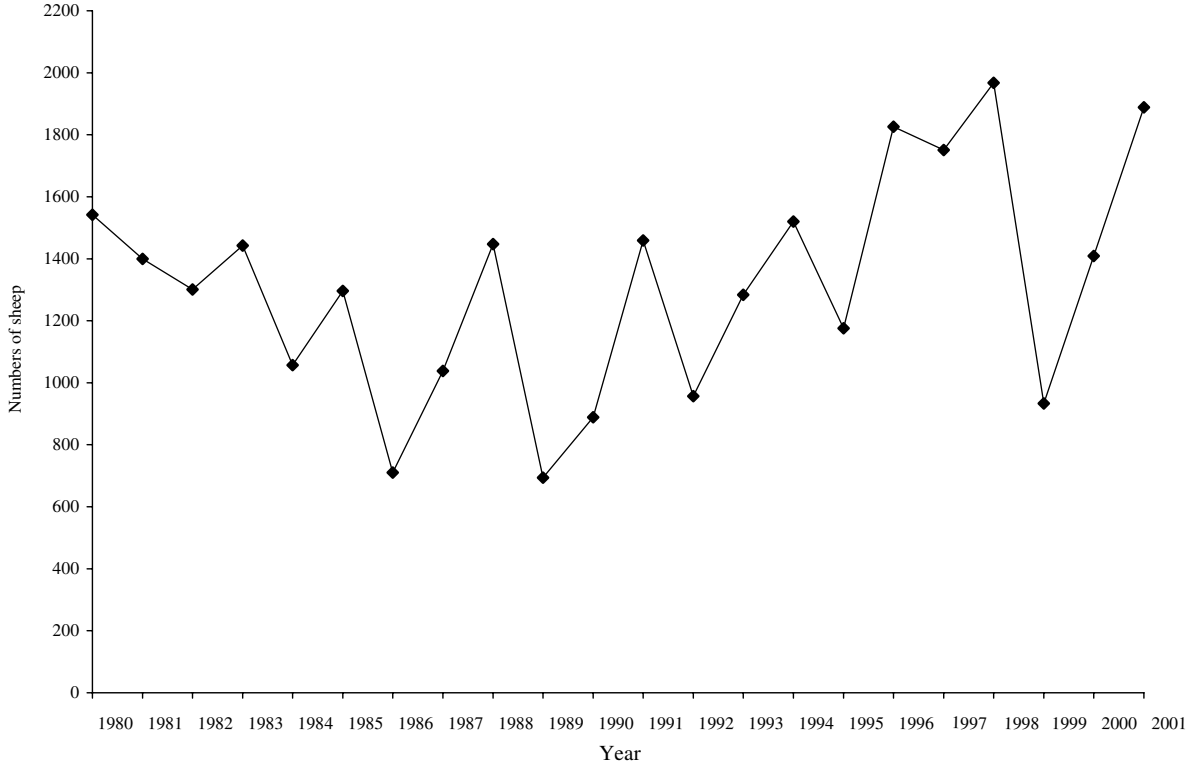

Figure 1 Numbers of Soay sheep on the island of Hirta, St Kilda, between 1980 and 2001.

Materials and methods

Study area and population sampling

Data are from the Village Bay study area that represent about 30% of the total island flock of Hirta and reflects the demographic processes in the whole island population (Coulson *et al*, 1999). Since 1985, over 95% of lambs born in the study area have been caught, individually tagged and sampled for genetic analyses shortly after birth. Every summer, about 65% of the study area population is caught and animals that have not been previously handled are tagged and sampled for genetic analyses. We used samples collected in each year between 1988 and 2000. Demographic crashes occurred during the winters 1988/1989, 1991/1992, 1994/1995 (a small crash) and 1998/1999. Samples correspond to the standing population defined in Coltman *et al* (2003), that is, only individuals that were 1 year of age or older, and that have been seen at least three times during the spring and summer censuses were considered. Census data and genetic studies have revealed that the Village Bay Soay sheep population is subdivided into three stable subunits called hefts (namely Central, South-western and Eastern hefts), corresponding to group of individuals, regardless of sex or age, that utilize the same resources in space, and that are genetically differentiated (Coulson *et al*, 1999; Coltman *et al*, 2003). Counts of gastrointestinal nematode eggs in fecal samples vary systematically between individuals located in different hefts, with individuals from the Eastern heft exhibiting larger FECs than those from the South-western or the Central hefts (Wilson *et al*, 2003).

Genotyping

We used genotype data obtained at six loci for all sampling dates between 1988 and 2000. There is one allozyme locus (Transferrin: TRF) and five microsatellite loci (OLADRB3, OarCP26, OarFCB304, MAF35 and MAF45). They have been shown to assort independently. They did not exhibit evidence of departure from Hardy–Weinberg equilibrium. These data have previously been published in Bancroft *et al* (1995), Pemberton *et al* (1996), Paterson *et al* (1998) and Coltman *et al* (1999, 2003). All of these loci are supposed to be neutral (TRF, OarCP26, OarFCB304, MAF35 and MAF45) except the locus OLADRB3, located in the MHC class II, within an intron adjacent to the exon 2 of the gene DRB that is involved in antigen presentation. Paterson *et al* (1998) have shown that there is a strong correlation between the microsatellite length variation and the exon sequence polymorphism, which indicates that the microsatellite analysis is a viable approach to assay genetic variation at the exon. Moreover, evidence of balancing selection acting on this microsatellite (Paterson, 1998) and association between OLADRB3 genotype and nematode FEC and survival have been detected previously (Paterson *et al*, 1998). The FEC, estimated per individual, is correlated with parasite burden (Gulland, 1992; Wilson *et al*, 2003) and reflects parasitic load. The MHC microsatellite OLADRB3 is thus a good candidate with which to study balancing selection acting on the MHC genes and driven by parasitism.

Within heft polymorphism over space and time

We first aimed at investigating whether the magnitude of variation in genetic polymorphism between hefts or between years was different for MHC and neutral loci. Fluctuations in genetic polymorphism were analysed within each heft across the 13-year survey. For all loci, genetic variation was described within heft for each sampling date using the allelic richness (A_{ll}) that measures the number of alleles per locus independently of the sample size (Petit *et al*, 1998), the mean observed heterozygosity (H_o), the unbiased gene diversity (H_e, see Nei, 1987) and the unbiased estimator $\hat{f}$ of Wright's inbreeding coefficient F_{IS} calculated according to Weir and Cockerham (1984). These parameters were calculated using FSTAT 2.9.3 (Goudet, 1995). Permutation tests based on 1000 permutations of populations between hefts (respectively, between years) were conducted using the software FSTAT 2.9.3 (Goudet, 1995) to detect spatial (respectively, temporal) fluctuations of the genetic polymorphism for MHC and neutral loci. Environmental heterogeneity in selection pressure is expected to lead to stronger variation of genetic polymorphism at MHC than at neutral loci, whereas balancing selection without local adaptation should lead to the opposite pattern. A similar magnitude of genetic polymorphism between hefts or years at MHC and neutral loci would indicate that balancing selection is too weak to overwhelm the effects of genetic drift and gene flow on the genetic polymorphism. The effect of balancing selection on each sample was also tested using the Ewens–Watterson neutrality test (Ewens, 1972) implemented in Arlequin 2.0 (Schneider *et al*, 2000). The sum of squares of allele frequencies (F) was compared to a null distribution of F generated by simulating random neutral samples (1000 replicates). The null hypothesis of neutrality was rejected if the observed values fell outside the 5–95% points of the distribution. An even distribution of allele frequencies is expected under balancing selection. The OLADRB3 MHC locus should thus exhibit a lower homozygosity than expected under neutrality.

Spatial genetic differentiation between hefts and its evolution over time

We compared the spatial differentiation observed at neutral and MHC loci to test for the influence of local selective constraints on population subdivision. Spatial genetic differentiation was analysed over the three hefts and for each pair of hefts for each locus and each date of sampling using a homogeneity test (Goudet *et al*, 1996) computed as an exact test with GENEPOP 3.1c and the sequential Bonferroni correction. The estimates $\hat{\theta}$ of F_{ST} were calculated according to Weir and Cockerham (1984) for each year of sampling over the three hefts and for each pair of hefts, per locus and over all neutral loci.

We first evaluated whether some of the five loci supposed neutral in this study could be identified as outliers. We applied a model-based approach to compare the observed F_{ST} values estimated at each locus to a null distribution of F_{ST} (conditional on heterozygosity) generated by a coalescent-based simulation model under a neutral model of population structure (Beaumont and Nichols, 1996). We used the program FDIST$_2$ developed by Beaumont and Balding (2004) to perform coalescent simulations using a symmetrical 15-island model of

population structure and the infinite alleles model. For each data set – which corresponds to three hefts and one sampling date – sample sizes were set equal to the median of the sample sizes and median estimates of F_{ST} were used to simulate data. Coalescent simulations were performed to generate 20 000 paired values of F_{ST} and heterozygosity. This curve was then used to compute the 0.975, 0.500 and 0.025 quantiles of the distribution of F_{ST} as a function of heterozygosity (Beaumont and Nichols, 1996). Loci with unusually high or low values of F_{ST} estimates are regarded as potentially under selection. The statistical significance of departures from the neutral expectation was assessed using two-tailed probabilities expressed as the proportion of 20 000 simulated values that were more extreme than the observed values. For each data set, the α-level was Bonferroni adjusted for multiple comparisons (0.05/number of loci).

We then chose to develop a model-free approach to evaluate whether the OLADRB3 MHC locus could be detected as an outlier under selection. The advantage of this empirical approach is that outlier detection is not biased by model-based assumptions about population structure and history (Storz, 2005). Moreover, following the suggestions of Beaumont and Nichols (1996), we did not apply their model-based approach as it is mainly recommended for studies including a large number of independent loci (>20) and of subpopulations (>10). Then, the 95% confidence interval (CI) of the five neutral loci estimates was calculated using 1000 bootstraps over loci using GENETIX 4.04 (Belkhir, 1996–2004). F_{ST} values estimated at OLADRB3 and over all the neutral loci were compared for each sampling date. MHC values outside the 95% CIs were considered significantly different from estimates derived from neutral loci (Weir, 1996).

Under the hypothesis of spatially fluctuating selection acting on the MHC, the extent of population subdivision at OLADRB3 is expected to exceed that observed at the five neutral markers (Muirhead, 2001; Bernatchez and Landry, 2003). Indeed, fluctuating selection can occur through a difference in the selective advantage of MHC alleles among environments. Selection thus promotes the maintenance of distinct allelic lineages in subpopulations (Jeffery and Bangham, 2000). Moreover, the selective advantage of alleles in a given environment (local adaptation) may fluctuate in time. Under this hypothesis of transitory local adaptation, the spatial genetic differentiation estimated at OLADRB3 compared to neutral loci would provide different patterns depending on the year considered. Considering the hypothesis of balancing selection without local adaptation, the spatial genetic differentiation between hefts should be significantly reduced at OLADRB3 compared with the five neutral loci, whatever the year considered (Schierup *et al*, 2000). Finally, an absence of significant differences of spatial differentiation between MHC and neutral loci would reveal that balancing selection does not overwhelm the effects of genetic drift and gene flow on population subdivision.

Within heft temporal genetic differentiation

We compared the trends of temporal genetic differentiation at neutral and MHC loci to test for the influence of temporal changes in selection pressures on population divergence. Temporal genetic differentiation of the standing population was analysed for each locus and heft over all pairs of years using a homogeneity test (Goudet *et al*, 1996) computed as an exact test with GENEPOP 3.1c and the sequential Bonferroni correction. The estimator $\hat{\theta}$ of F_{ST} (Weir and Cockerham, 1984) was calculated for each locus for each pair of years. The null hypothesis of independence between temporal (number of years separating two samples) and genetic distance was tested against a positive correlation using Mantel tests (isolation by temporal distance, equivalent to an isolation by geographical distance). This was assessed with the program 1.2 IBD (Bohonak, 2002). F_{ST} estimates, computed for pairs of populations separated by 1–12 years, were regressed on the number of years between samples to determine whether there was a trend toward increasing genetic differentiation with time (Walker and Levy, 2001). Note that negative F_{ST} estimates were set to zero. The slope and the intercept of this relationship were estimated using reduced major regression axis. Their 95% CIs were calculated using jacknife over independent population pairs as it is the recommended approach (Bohonak, 2002). The comparison of these CIs allows the detection of differences in the trends of temporal differentiation among the three hefts and between the MHC and the neutral loci. If selection varies in direction through time, the temporal genetic differentiation within a heft should increase more quickly than under neutrality. Under this hypothesis of temporal local adaptation, the time required to promote temporal differentiation at OLADRB3 should be lower than observed at the neutral loci. On the one hand, because the three subpopulations are not at migration-drift equilibrium, comparing the temporal genetic differentiation within each heft may provide information about the existence of environmental heterogeneity in the selection pressure acting on OLADRB3. Different rates of temporal evolutionary changes of the sheep population in the three hefts would reveal local selective constraints. Stronger trends in temporal genetic differentiation are expected at OLADRB3 within the Eastern heft where the intensity of selection pressure is more important. On the other hand, assuming that the same MHC alleles are selected over successive demographic crashes, the temporal genetic divergence of a population estimated over several fluctuations at the MHC locus OLADRB3 should be smaller than the temporal differentiation estimated at neutral loci that are affected by other microevolutionary processes such as drift and migration. Finally, similar trends of genetic temporal differentiation at MHC and neutral loci would reveal that selection does not overwhelm genetic drift.

Results

Within heft polymorphism over space and time

Permutation tests were first performed between hefts on polymorphism parameters to test for spatial variation. At OLADRB3, a significant spatial variation of the allelic richness and of the gene diversity was detected over the 13-year survey (A_{ll}: $P<0.001$; H_e: $P<0.001$). A similar spatial variation of these two measures of genetic polymorphism was observed at the five non-MHC loci (A_{ll}: $P<0.05$; H_e: $P<0.05$ for each of the five loci). The two other estimates of genetic polymorphism, namely

H_o and F_{IS}, show no significant spatial variation at OLADRB3 and different patterns according to the non-MHC locus considered. A significant spatial variation was observed at OarCP26 (H_o: $P<0.001$; F_{IS}: $P=0.017$) and MAF35 (H_o: $P<0.001$; F_{IS}: $P=0.042$), whereas no significant variation was detected at OarFCB304, MAF45 and TRF. Second, permutation tests were performed between years of sampling, to test for temporal variation of genetic polymorphism. No significant variation of the genetic polymorphism was detected throughout the 13-year temporal survey at any of the loci. The Ewens–Watterson neutrality test did not detect a signal of balancing selection (across all hefts and dates), whatever the locus considered.

Spatial genetic differentiation between hefts and its evolution over time

The results from the analyses of spatial differentiation are presented in Table 1. The model-based approach developed using FDIST$_2$ did not detect any outliers among the five loci supposed to be neutral in this study (Table 1, Figure 2). Whatever the data set considered, the expected distribution of F_{ST} generated under the neutral

Table 1 Values of F_{ST} estimated over all hefts for each year of sampling per locus and over all loci except OLADRB3

Year	*MHC locus OLADRB3*	*Neutral loci*					*All neutral loci*
		TRF	*OarFCB304*	*OarCP26*	*MAF35*	*MAF45*	
1988	*0.007* (0.019)	*0.014* (4×10^{-4}) $P_F=0.943$	0.003 (0.070) $P_F=0.541$	0.003 (0.390) $P_F=0.595$	*0.011* (0.034) $P_F=0.569$	0.001 (0.267) $P_F=0.123$	*0.006* (7×10^{-4})
1989	*0.019* (0.007)	0.005 (0.063) $P_F=0.475$	0.013 (0.089) $P_F=0.738$	*0.026* (0.005) $P_F=0.919$	0.010 (0.310) $P_F=0.573$	−0.004 (0.771) $P_F=0.018$	*0.009* (0.023)
1990	*0.011* (0.050)	*0.018* (0.004) $P_F=0.883$	0.000 (0.151) $P_F=0.203$	*0.014* (0.023) $P_F=0.771$	*0.034* (0.020) $P_F=0.847$	−0.006 (0.961) $P_F=0.012$	*0.009* (0.002)
1991	*0.011* (0.003)	*0.010* (0.009) $P_F=0.549$	*0.009* (0.021) $P_F=0.505$	*0.023* (2×10^{-4}) $P_F=0.917$	*0.014* (0.008) $P_F=0.703$	0.000 (0.312) $P_F=0.065$	*0.011* ($<10^{-4}$)
1992	*0.011* (0.005)	*0.008* (0.048) $P_F=0.586$	*0.007* (0.034) $P_F=0.525$	0.005 (0.066) $P_F=0.573$	*0.028* (0.002) $P_F=0.893$	0.001 (0.214) $P_F=0.130$	*0.007* ($<10^{-4}$)
1993	*0.004* (0.013)	0.002 (0.257) $P_F=0.382$	*0.010* (0.002) $P_F=0.685$	0.004 (0.053) $P_F=0.523$	*0.021* (0.002) $P_F=0.900$	0.003 (0.217) $P_F=0.304$	*0.007* ($<10^{-4}$)
1994	*0.008* (0.006)	0.003 (0.077) $P_F=0.294$	*0.016* (2×10^{-4}) $P_F=0.833$	0.001 (0.301) $P_F=0.149$	*0.018* (0.003) $P_F=0.895$	0.005 (0.056) $P_F=0.572$	*0.008* ($<10^{-4}$)
1995	*0.006* (0.023)	0.006 (0.118) $P_F=0.609$	*0.002* (0.015) $P_F=0.409$	0.003 (0.113) $P_F=0.673$	*0.013* (0.030) $P_F=0.709$	0.005 (0.090) $P_F=0.617$	*0.006* (10^{-4})
1996	0.002 (0.203)	*0.008* (0.016) $P_F=0.738$	*0.006* (3×10^{-4}) $P_F=0.679$	*0.006* (0.003) $P_F=0.780$	*0.009* (0.006) $P_F=0.741$	−0.002 (0.619) $P_F=0.018$	*0.006* ($<10^{-4}$)
1997	−0.001 (0.541)	*0.008* (0.016) $P_F=0.767$	*0.005* (0.002) $P_F=0.617$	0.002 (0.057) $P_F=0.437$	*0.018* (3×10^{-4}) $P_F=0.928$	0.000 (0.292) $P_F=0.135$	*0.006* ($<10^{-4}$)
1998	−0.002 (0.933)	*0.013* (2×10^{-4}) $P_F=0.912$	0.001 (0.090) $P_F=0.233$	*0.004* (0.010) $P_F=0.494$	*0.018* (0.002) $P_F=0.867$	0.001 (0.246) $P_F=0.109$	*0.007* ($<10^{-4}$)
1999	0.002 (0.291)	*0.010* (0.003) $P_F=0.609$	*0.007* (0.037) $P_F=0.532$	*0.012* (0.003) $P_F=0.765$	*0.014* (0.116) $P_F=0.912$	0.000 (0.090) $P_F=0.054$	*0.008* ($<10^{-4}$)
2000	−0.002 (0.602)	0.008 (0.079) $P_F=0.877$	2×10^{-4} (0.131) $P_F=0.360$	*0.010* (0.006) $P_F=0.968$	−0.002 (0.640) $P_F=0.135$	*−0.001* (0.010) $P_F=0.042$	*0.003* (0.001)

The significance of these values, indicated in parentheses, was tested according to the exact test and the Fisher's method. Italic values indicate significant exact tests. P_F is the two-tailed probability for one supposed neutral locus to depart from the neutral expectation provided by simulation (see text for details).

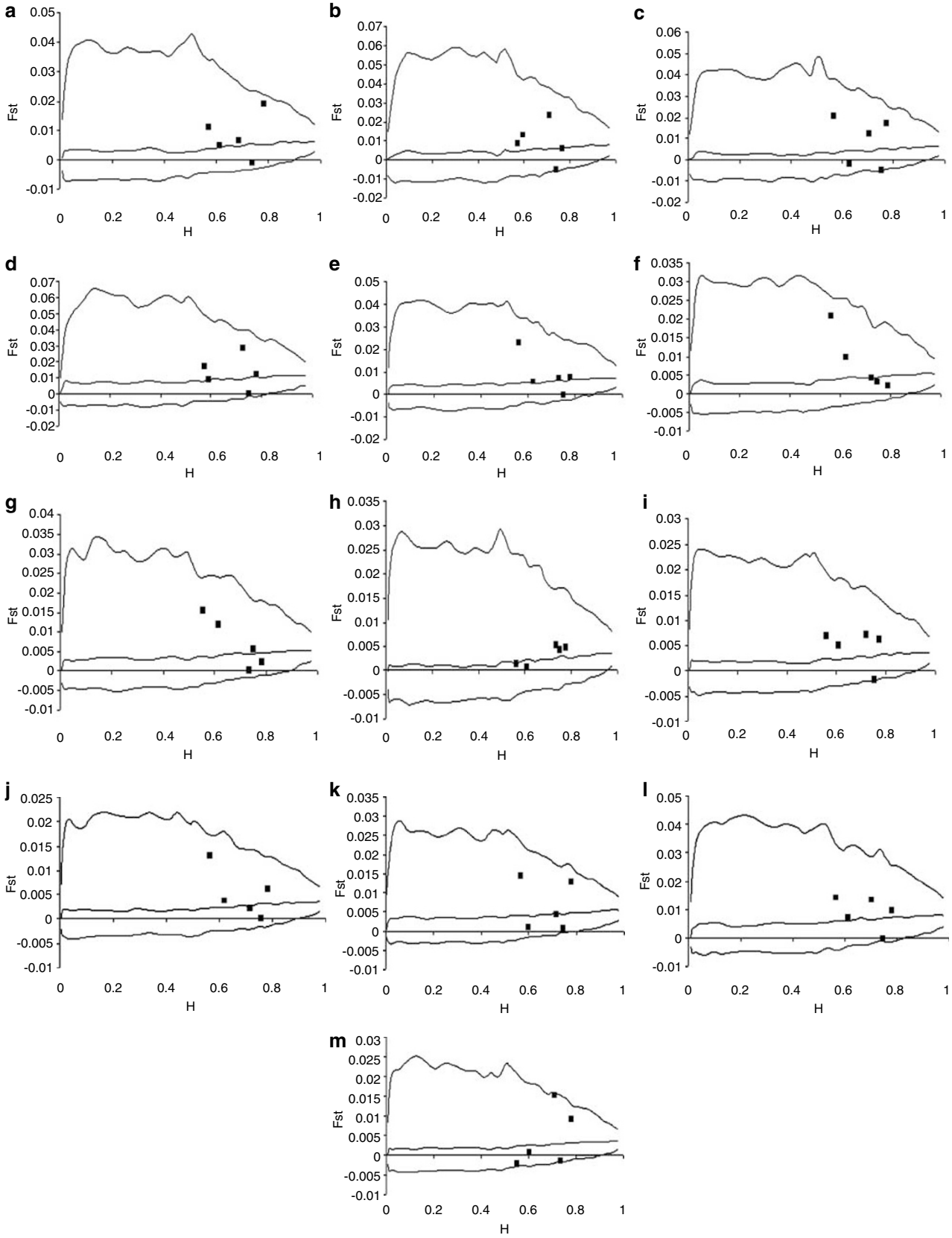

Figure 2 Estimated F_{ST} values from the five supposed neutral loci plotted as a function of heterozygosity (H). Results are shown for each data set corresponding to one sampling date and three hefts: A to M refers to the year 1988 to 2000. Lines denote the 0.975, 0.500 and 0.025 quantiles of the distribution of F_{ST} against heterozygosity estimated from 20 000 simulations (see text for details).

model selected as a function of heterozygosity gave a good fit of the data. F_{ST} values for all supposed neutral loci lay within the 0.025 and 0.975 quantiles of the calculated distribution (Figure 2).

The exact tests and Bonferroni correction performed over the three hefts at OLADRB3 indicated a significant spatial differentiation for all years between 1988 and 1995, and an absence of significant spatial differentiation between 1996 and 2000 (Table 1, Figure 3). The results observed at neutral loci showed different patterns. When combining all neutral loci, all exact tests were highly significant ($P<0.001$), whichever year was considered (Table 1, Figure 3). This is a first line of evidence of a difference in magnitude of spatial genetic differentiation at MHC and neutral loci between 1996 and 2000. This is confirmed by the F_{ST} estimates. Between 1988 and 1995, the values of F_{ST} estimated at OLADRB3 ranged between 0.004 and 0.019. Considering the 95% CI, these values were not significantly higher than the values of F_{ST} estimated over all the five neutral loci (range: 0.006–0.011) at the same sampling dates (Figure 3). Between 1996 and 2000, the values observed at OLADRB3 were very low or null (range: −0.002 to 0.002), whereas the values of F_{ST} estimated over all the five neutral loci remained higher and comparable to the previous years (range: 0.003–0.008). The OLADRB3 F_{ST} values were significantly lower (based on the CI; Figure 3); indeed, the OLADRB3 value was lower in all five single-locus estimates from the neutral loci. When considering pairwise comparison of hefts, different patterns were observed through time. Briefly, between the Eastern and the South-western hefts or between the Eastern and the Central hefts, both the exact tests and the F_{ST} estimates indicated that the spatial genetic differentiation estimated at OLADRB3 was significantly lower than the differentiation estimated at the five other loci from 1996 to 1999 (Figure 3). Indeed, after applying Bonferroni corrections, the exact tests were all significant when the five non-MHC loci were combined, while the exact tests concerning the MHC locus were never significant. Moreover, the OLADRB3 F_{ST} estimates were lower than the neutral F_{ST} estimates and fell outside the 95% neutral CI (Figure 3). The same pattern was observed in 1993 between the Eastern and the Central hefts (MHC $F_{ST}=-0.002$, P (F_{ST}) = NS; neutral loci, $F_{ST}=0.011$, P (F_{ST}) <0.05, 95% CI: 0.004–0.021) and in 1988 (MHC $F_{ST}=-0.004$, P (F_{ST}) = NS; neutral loci, $F_{ST}=0.005$, P (F_{ST}) $=0.04$, 95% CI: −0.002 to 0.013) and 1994 (MHC $F_{ST}=-5\times10^{-5}$, P (F_{ST}) = NS; neutral loci, $F_{ST}=0.008$, P (F_{ST}) <0.05, 95% CI: 7×10^{-4} to 0.016) between the Eastern and the South-western hefts. A significantly higher differentiation was observed at the MHC locus compared to the other loci in 1988 between the Eastern and the Central hefts (MHC $F_{ST}=0.016$, P (F_{ST}) <0.05; neutral loci, $F_{ST}=0.005$, P (F_{ST}) <0.05, 95% CI: -8×10^{-4} to 0.011) and in 1989 between the Eastern and the South-

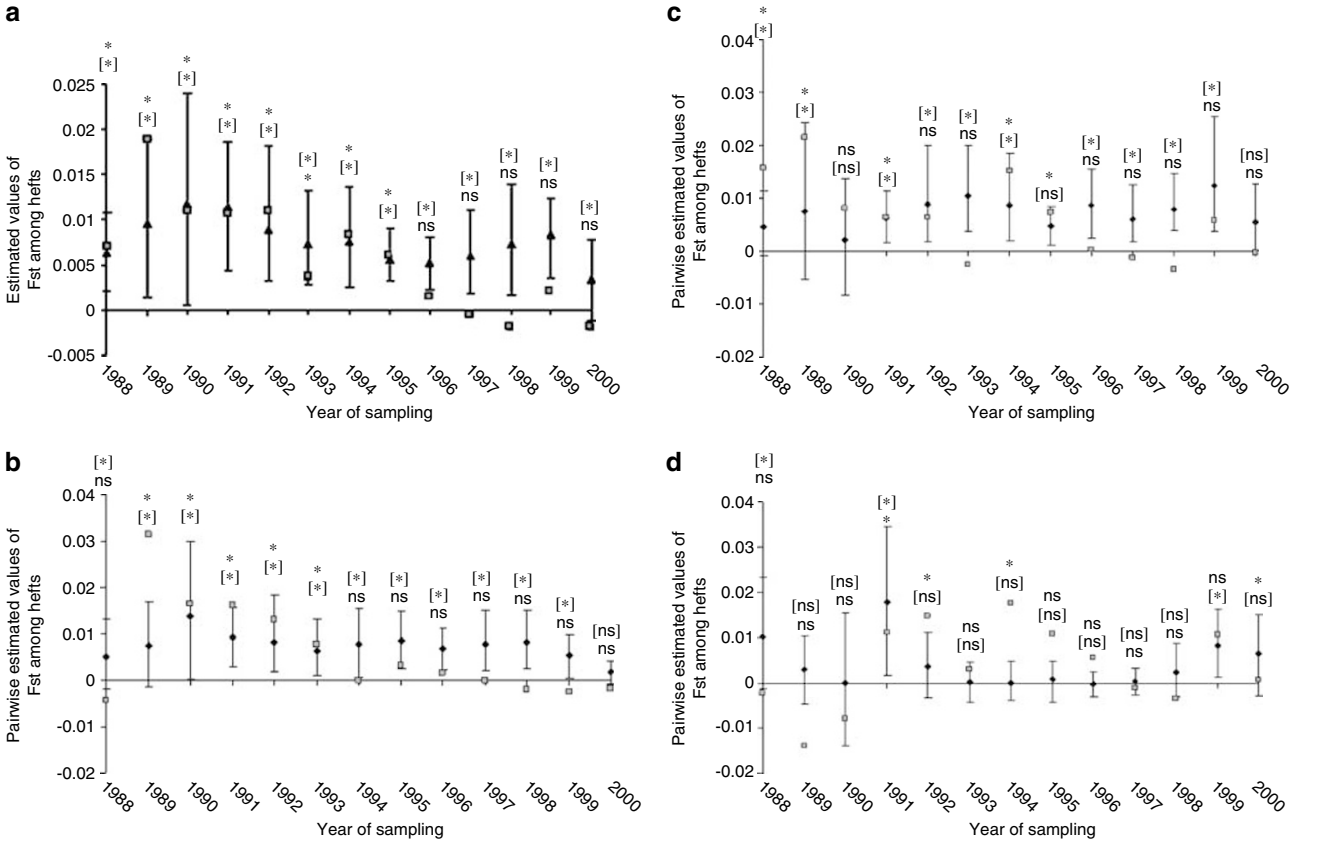

Figure 3 Comparison of F_{ST} values estimated over all hefts (**a**) or between the South-western and the Eastern hefts (**b**) the Central and the Eastern hefts (**c**) and the Central and the South-western hefts (**d**) for each year of sampling over all the neutral loci (black triangles) and at OLADRB3 only (grey squares). Error bars indicate the 95% CI of the F_{ST} values estimated using 1000 bootstraps over all loci except OLADRB3. Probabilities associated with the exact tests of spatial differentiation are indicated using the symbols ns for nonsignificant values and * for significant values. Symbols inside brackets correspond to the exact tests associated with the five non-MHC loci.

western hefts (MHC $F_{ST} = 0.031$, P (F_{ST}) < 0.05; neutral loci, $F_{ST} = 0.007$, P (F_{ST}) < 0.05, 95% CI: -0.001 to 0.017) (Figure 3). The patterns of spatial differentiation observed between the Central and the South-western hefts at OLADRB3 and at the five neutral loci were less variable. Following the exact tests and the 95% CIs of neutral F_{ST} estimates, no significant differences were observed, except in 1988 when the spatial differentiation was stronger at the neutral loci (MHC $F_{ST} = -0.002$, P (F_{ST}) = NS; neutral loci, $F_{ST} = 0.010$, P (F_{ST}) < 0.05, 95% CI: -0.001 to 0.023) and in 1992 (MHC $F_{ST} = 0.015$, P (F_{ST}) < 0.001; neutral loci, $F_{ST} = 0.004$, P (F_{ST}) = NS, 95% CI: -0.003 to 0.011) and 1994 (MHC $F_{ST} = 0.018$, P (F_{ST}) < 0.001; neutral loci, $F_{ST} = -5 \times 10^{-5}$, P (F_{ST}) = NS, 95% CI: -0.004 to 0.005) when the opposite pattern was observed (Figure 3).

Within heft temporal genetic differentiation

The five neutral loci exhibited no pattern of genetic divergence through time when considering the Central heft (Mantel test: $P = 0.085$, slope $= 1.2 \times 10^{-3}$, 95% CI: -2.9×10^{-5} to 2.8×10^{-3}) and a highly significant isolation by temporal distance within the South-western and the Eastern hefts (Mantel test: South-western heft: $P = 0.025$, slope $= 6.6 \times 10^{-4}$, 95% CI: -1.2×10^{-4} to 1.8×10^{-3}; Eastern heft: $P = 0.001$, slope $= 1.9 \times 10^{-4}$, 95% CI: 1.8×10^{-5} to 4.3×10^{-4}). The comparison of the 95% CI of the slopes indicated that the trends toward increasing genetic differentiation with time were not significantly different in these two hefts. The locus OLADRB3 showed similar results (Figure 4). No significant pattern of temporal genetic divergence was observed in the Central heft (Mantel test: $P = 0.157$, slope $= 1.8 \times 10^{-4}$, 95% CI: -4.3×10^{-4} to 4.4×10^{-4}), whereas a highly significant isolation by temporal distance was observed in the South-western (Mantel test: $P = 0.009$, slope $= 1.2 \times 10^{-3}$, 95% CI: 4.6×10^{-4} to 2.1×10^{-3}) and in the Eastern hefts (Mantel test: $P = 0.001$, slope $= 1.0 \times 10^{-3}$, 95% CI: 7.4×10^{-4} to 1.3×10^{-3}). The trends of temporal genetic divergence are also similar in the Eastern and the South-western hefts at the OLADRB3 locus. However, the 95% CI of the slope of the regression also indicates that in the Eastern heft, the temporal genetic differentiation is significantly higher at the MHC locus than at the five neutral loci (Figure 4).

Discussion

Previous studies conducted on the MHC in the Soay sheep population of St Kilda revealed evidence of balancing selection (Paterson, 1998) and of negative frequency dependence when considering lambs and yearlings, some MHC alleles being associated, in interaction with weight, with differences in juvenile survival and resistance to *T. circumcincta* (Paterson *et al*, 1998). However, the long-term temporal and spatial genetic data from this natural population had not been analysed with the aim of studying the mechanism of balancing selection. On the other hand, evidence of fluctuating selection as a mechanism of balancing selection in mammals had never been observed using comparison of spatial genetic differentiation patterns (Bernatchez and Landry, 2003), and no temporal genetic survey of mammal populations has previously been analysed in this way. This data set thus represented a unique opportunity to test for differences in selective advantage of MHC alleles or genotypes in space and time in a mammalian population.

Some of the genetic patterns observed at the MHC locus were not different from those observed at the non-MHC loci, indicating that the effects of balancing selection do not overwhelm those of genetic drift and migration. First, we found no evidence of balancing selection by contrasting the spatial and temporal variation of genetic polymorphism estimated at the MHC and the five non-MHC loci or by performing neutrality tests. However, these tests may not be appropriate in the case of fluctuating selection as they rely on the assumption that the populations tested are at equilibrium and have historically been of constant size (see for a review Garrigan and Hedrick, 2003). The tests, thus, suffer from low power and make inference about selection difficult. Second, the spatial genetic differentiation estimated over the three hefts between 1988 and 1995 was similar at the MHC and at the non-MHC loci. Finally, the general patterns of differentiation over time observed in the three hefts were globally equivalent at the MHC and at the non-MHC loci: a significant temporal divergence was observed in the Eastern and South-western hefts but not in the Central heft. Such absence of indicators for balancing selection has been observed in several other studies of mammalian populations (eg Bernatchez and Landry, 2003; Seddon and Ellegren, 2004). However, other parts of our results have given some credence to the hypotheses of balancing selection and environmental fluctuation in selective pressure.

Balancing selection and selective equivalence in space

Between 1996 and 2000, contrasting the patterns of overall genetic differentiation estimated at OADRB3 and at the five non-MHC loci revealed balancing selection at the MHC with no differences in local selective constraints. Indeed, a significantly weaker genetic differentiation was observed over all hefts at the MHC compared to the neutral loci. These different patterns of spatial differentiation at the MHC and non-MHC loci were also observed in several years between pairs of hefts. In 10 out of 11 cases, the Eastern heft was involved. The most plausible is the selection of the same alleles or genotypes in the different hefts, which are thus kept in more equal frequencies than at neutral loci (Hedrick, 1999). A similar result had previously been observed in the San Nicolas Island fox populations (Aguilar *et al*, 2004). Another possibility involves the hypothesis of negative frequency dependence at the MHC, incoming immigrant alleles or genotypes in a subpopulation being selected for (Schierup *et al*, 2000). This latter hypothesis is quite unlikely in the Soay sheep population, as the migration rate between the three hefts is quite high (Coltman *et al*, 1999) and because fluctuations of allele frequencies did not provide evidence of rare incoming alleles in hefts.

Balancing selection, heterogeneity in selection pressure

An interesting result emerging from this study is the existence of temporal heterogeneity in the intensity and the direction of selection. First, whatever the spatial

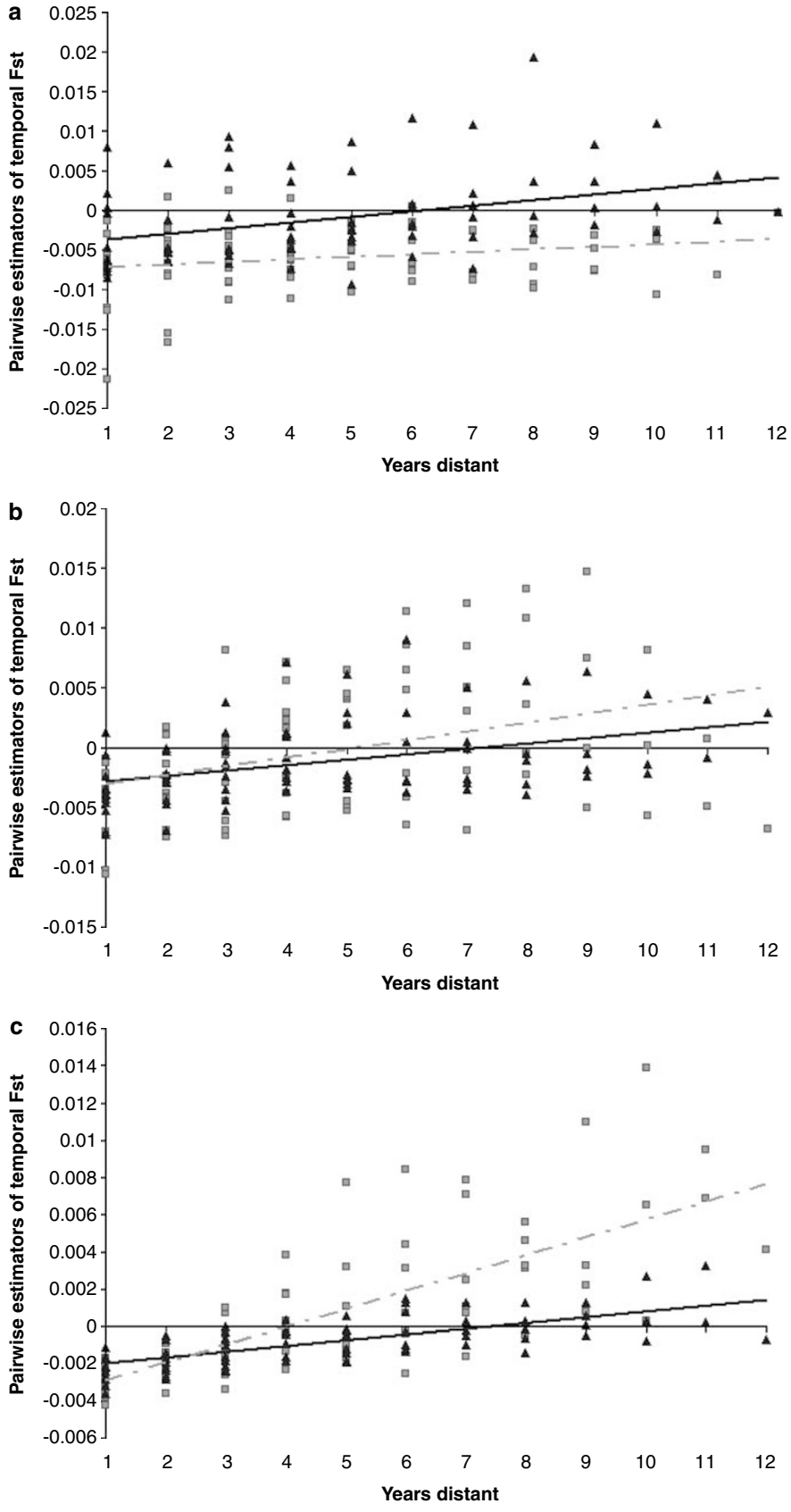

Figure 4 Genetic differentiation of the standing population of St Kilda Soay sheep between pairs of sampling dates. Estimates of pairwise temporal genetic differentiation at OLADRB3 (grey squares, dotted line) and at the five neutral loci combined (black triangles, thick line) are plotted against the number of years between two samples. These linear regressions are shown for the Central (**a**), South-western (**b**) and Eastern (**c**) hefts.

differentiation considered, meaning over all hefts or between pairs of hefts, there is variation from year to year of the patterns observed at the MHC and the five non-MHC loci. This variation concerned both the existence and absence of balancing selection, as observed when looking at the overall spatial differentiation after 1996 or before 1995, and the existence and absence of spatial heterogeneity of selection pressure, as observed when comparing pairs of hefts. The different levels of subdivision observed at the MHC locus do not appear to be artefacts related to the different amounts of polymorphism observed among loci. This conclusion follows from two considerations: markers with higher heterozygosity are expected to have lower F_{ST} estimates (Hedrick, 1999); and there is no significant variation in heterozygosity through time, whichever locus is considered. Second, we have satisfying evidence of a strong temporal variation in the direction of the selection pressure in the Eastern heft. Indeed, the temporal genetic divergence observed within this heft at the MHC is significantly higher than at the neutral loci. A similar result was observed by Westerdahl *et al* (2004) in adult great reed warblers. The significant variance in MHC allele frequency between successive cohorts was likely to be explained by fluctuating selection pressures from pathogens and parasites.

The other important result of this study is the demonstration of spatial heterogeneity in the intensity and the direction of selection pressure. First, the comparison of the spatial genetic differentiation between each pair of hefts supported this hypothesis. In four sampling years, the spatial genetic differentiation estimated at the MHC was significantly stronger than the differentiation estimated over the five neutral loci. This supports the hypothesis of balancing selection driven by differences in selective advantages among hefts (Jeffery and Bangham, 2000). Some local adaptations may exist at OLADRB3 at the scale of the Village Bay area, although, if so, they are very transitory. A similar result had previously been observed in salmon populations where the stronger spatial differentiation observed at MHC than microsatellite loci was explained by differential selection driven by parasites across habitats within spawning areas (Landry and Bernatchez, 2001). However, this report is the first time that such result is observed in mammals; indeed, previous studies conducted in mammalian populations have revealed no differences or lower differentiation at MHC than at the neutral loci (eg Gutierrez-Espeleta *et al*, 2001; Aguilar *et al*, 2004). Second, the time required to achieve temporal differentiation at the MHC was significantly lower than at the neutral loci. This contrasting pattern indicates that the selection acting on the MHC was stronger in the Eastern than in the two other hefts. This conclusion supports the reports of a higher intensity of parasite pressure in the Eastern heft (Wilson *et al*, 2003) affecting its differentiation over time.

Plausible causes of spatial or temporal fluctuations in parasite pressure

Although this study concerns a small geographical scale (175 ha), there is known to be spatial variation in parasite intensity between hefts, and in particular FECs from the Eastern heft where higher (Wilson *et al*, 2003). This pattern is partly maintained by variation in the density of sheep in the three hefts. Food quality and availability are known to vary temporally and spatially between hefts (Boyd and Jewell, 1974) and to interact with immunity and survivorship, as indicated by the strong associations observed between MHC and individual weight (Paterson *et al*, 1998). Local variation in the vegetation in space and time could mediate parasite selection pressure. Coevolution between the sheep MHC and the strongyle populations within each heft could, therefore, plausibly explain the signals of transitory local adaptation detected in this study.

Conclusions

By conducting population genetics analyses on the long-term survey data set obtained for the Soay sheep population of St Kilda, our study suggests that fluctuation in selection in space and time is probably an important mechanism driving balancing selection at the MHC, although some evidence for frequency dependence had previously been observed. Further analyses are required in the Eastern heft to confirm whether frequency-dependent selection involving a few alleles and/or overdominance explains the high temporal divergence observed at the MHC locus OLADRB3 over this 13-year survey. Moreover, understanding the mechanisms responsible for the selection remains an open question. More knowledge of the population dynamics and genetics of the nematode *T. circumcincta*, the main macroparasite implicated in the Soay sheep mortality, are now needed. Studies should aim to determine whether fluctuating selection is a consequence of parasite prevalence or virulence, or indirectly through the variation in the fitness costs of infection as resource availability changes. In addition, further theoretical work exploring the relative influence of overdominance, frequency-dependence and fluctuating selection on population subdivision and divergence are now required to fully understand the evolution of MHC diversity in the Soay sheep population.

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