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Extensive protein and microsatellite variability in an isolated, cyclic ungulate population

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We investigated polymorphism at protein and microsatellite DNA loci in an isolated, unmanaged and cyclic population of Soay sheep on the island of Hirta, St. Kilda. Extensive molecular variation was revealed at both protein loci (mean heterozygosity 7.78 per cent) and microsatellite loci (mean heterozygosity 50.93 per cent). Typically, large mammals possess limited protein variation and we were surprised to observe such a level of protein heterozygosity, particularly considering the genetic history of the Hirta population. Indeed, compared to other mammals, Soay sheep lie within the top 17 per cent of the distribution of average protein heterozygosities. We discuss the level of protein heterozygosity in the context of other mammalian species, other breeds of sheep and the genetic history of Soay sheep. Possible explanations for a large average and interlocus variance in protein heterozygosity are proposed. Although little data are available from other studies to compare with microsatellite DNA variability in this population, we discuss the potential application of microsatellite markers to interpopulation and interspecific genetic studies.

Keywords: allozymes, bottlenecks, microsatellites, molecular variation, natural population, sheep.

Introduction

Since the development of enzyme electrophoresis (Hunter & Markert, 1957) numerous natural populations of organisms have been investigated for genetic variation at a range of protein loci (Nevo *et al.*, 1984). More recently, many new DNA-based methods, usually offering much greater resolution of differences between individuals and populations, have become available. These include mitochondrial DNA variation (Avise, 1986; Cann *et al.*, 1987), restriction fragment length polymorphism (RFLP) (Barrie *et al.*, 1981; Quinn *et al.*, 1987), minisatellite DNA variation, including DNA fingerprinting (Jeffreys *et al.*, 1985), random amplified polymorphic DNAs (RAPDs) (Welsh & MacClelland, 1990; Williams *et al.*, 1990) and microsatellite DNA variation (Litt & Luty, 1989; Weber & May, 1989; Tautz, 1989). Whichever genetic technique is utilized and in whatever context it is applied, these approaches almost always generate debate on the evolutionary forces affecting the observed distribution of genetic variability found.

Forces believed to be involved in determining allelic variability at any particular marker in a population include genetic drift, selection and sequence-specific events within the genome. Genetic drift is (nowadays) perhaps the least controversial. The way in which genetic variation is lost by drift in small populations has been described theoretically (Wright, 1931; Crow & Kimura, 1970; Nei *et al.*, 1975) and confirmed both experimentally (Sing *et al.*, 1973) and by observation of low genetic variability in species with documented population bottlenecks, such as the Northern elephant seal (*Mirounga angustirostris*) (Bonnell & Selander, 1974; Hoelzel *et al.*, 1993), and Ngorongoro Crater lions (*Panthera leo*) (Wildt *et al.*, 1987). Such is the influence of this belief, that studies which reveal low levels of genetic variation in a population commonly infer that a population bottleneck has occurred, e.g. cheetah (*Acinonyx jubatus*) (O'Brien *et al.*, 1983, 1987) and fallow deer (*Dama dama*) (Pemberton & Smith, 1985).

There are reasons for believing that the consequences of population bottlenecks for genetic variabi-

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lity are not yet fully understood. First, in experimental studies, though molecular variation may be reduced in the predicted manner, quantitative variation is not, and may even increase (Bryant *et al.*, 1986), a result thought to come about through disruption of balanced groups of genes (Carson, 1990). Second, though the expected loss of molecular variation may take place in theoretical or experimental bottlenecks when the researcher removes individuals at random, this may not be true of natural bottlenecks (Carson, 1990). Many natural populations decline because they are subject to changed environmental conditions, so at the very same time that genetic loss by drift may be expected, those genotypes more suited to changed conditions may be favoured. It seems likely that the genetic outcome of bottlenecks in natural populations will vary from case to case and that empirical studies are required to establish general patterns in this area.

In this paper we describe an investigation of genetic variability in Soay sheep (*Ovis aries*) living in the remote Scottish archipelago of St. Kilda. The history of this population, which combines isolation with frequent population crashes, and its polygynous mating system, led us to expect low levels of molecular variation in the population. Surprisingly, the population retains extensive genetic variation at both protein and microsatellite DNA loci. As a result, we suggest that the population crashes, far from removing genetic variation from the population, may in some way be involved in maintaining variation.

Materials and methods

Study population

The St. Kilda group consists of four islands and numerous stacks lying 60 km west of the Outer Hebrides, Scotland. Soay sheep are a relic of prehistoric farming practices and although unrecorded, their introduction to St. Kilda may have occurred as long ago as Viking times (Boyd & Jewell, 1974). By historical times, Soay sheep were restricted to the island of Soay (99 ha) after which they are named. Though harvested for wool and meat, the sheep were not intensively managed. We cannot exclude the possibility that rams from improved breeds were released onto Soay from time to time. In the summer of 1932, after evacuation of humans and all their domestic stock, 107 sheep were captured on Soay (20 rams, 44 ewes, 21 ewe lambs and 22 ram lambs (all of which were castrated)) and released onto the larger adjacent island of Hirta (637 ha) (Boyd, 1953). By 1952, the unmanaged Hirta population had reached an estimated size of 1114 sheep (Boyd, 1953) and since regular censusing began in 1955, has experienced frequent population crashes, sometimes occurring as often as one year in three, in which up to 70 per cent of the population dies (Grubb, 1974; Clutton-Brock *et al.*, 1991, 1992; Grenfell *et al.*, 1992). These population dynamics probably result from the fact that in a single breeding season the sheep population is able to increase far above the winter carrying capacity of the island (Grenfell *et al.*, 1992); increased burdens of parasitic nematodes may also contribute to the severity of crashes (Gulland, 1992; Gulland *et al.*, 1993). Crash mortality is heavily biased towards young animals and males, leading to a female-biased adult sex ratio (Clutton-Brock *et al.*, 1991, 1992). Males probably die more because they enter the winter in poor condition after the November rut (Stevenson, 1994).

For a period in the 1970s (Jewell *et al.*, 1974) and since 1985 (Clutton-Brock *et al.*, 1991, 1992; Grenfell *et al.*, 1992) the Soay sheep living in the Village Bay area of Hirta (a 170 ha bowl of land to the south-east of the island which holds around 30 per cent of the total population) have been intensively monitored. An interdisciplinary study aims to understand the population dynamics and identify the causes and consequences of variation in individual reproductive success. At present, 90 per cent of lambs born in the study area are tagged at birth and some of the remainder in a summer roundup of the population. Lambing, rut behaviour and mortality are monitored in detail.

To measure male reproductive success and to determine paternal genetic contributions to offspring success, accurate information on paternity of individual lambs is required. Since both sexes are highly promiscuous in the rut, a genetic method for identifying paternity is needed. The genetic study described here was initiated partly to fulfil this requirement and partly to determine the extent of molecular variability in the population and its fate during the periodic population crashes.

Although the history of the Soay sheep population (above) suggests that the Soay sheep might be impoverished with respect to genetic variation, there were some initial indications of genetic differentiation between individuals. First, Soays are phenotypically variable. They are polymorphic in coat colour, for which a two-locus model of inheritance has been proposed (Doney *et al.*, 1974), and they are polymorphic for horn type with polled, horned and scurred (having small, misshapen horns) individuals in the population (Doney *et al.*, 1974). Second, a previous brief study of blood groups in the population found three polymorphic systems (haemoglobin, transferrin and potassium type) (Hall, 1974).

Tissue sampling and preparation

Soay sheep were sampled whenever captured, either as newborn lambs in a summer catch-up or, for immigrant males, when tranquillized during the rut. We took a 4 mm ear punch prior to ear tagging and 2–10 mL of jugular blood. Since 1985 samples from over 1700 animals born or resident within the Village Bay study area have been collected.

Within 24 hours of collection, whole jugular blood was separated by centrifugation at 1000 ***g*** for 10 min. 1 mL of plasma was collected and the remaining plasma was discarded. Red and white cells were gently washed by mixing with 0.9 per cent saline and re-fractioned by a second 1000 ***g*** centrifugation. White cells were collected from the red cell–saline boundary and red cells from below it. The fractionated plasma, white cells and red cells, together with the ear punches, were individually labelled and frozen at −20°C until required.

Protein screening

To identify polymorphic proteins, we conducted a pilot survey at 34 soluble protein loci involving at least 20 and usually 40 randomly chosen individuals from the Hirta population for each locus. The protein loci screened are listed in Table 1. Initially, all proteins were screened by starch gel electrophoresis, following methods given in Ritter *et al.* (1974), Manlove *et al.* (1975), Harris & Hopkinson (1976) and Gyllensten *et al.* (1983).

Five polymorphic 'study' protein loci were subsequently screened over large numbers of individuals from the study population. For improved speed, four loci, haemoglobin (*β-Hb*), cytoplasmic isocitrate dehydrogenase (*Idh-1*), mitochondrial glutamate oxaloacetate transaminase (*Got-2*), and adenosine deaminase (*Ada*) were routinely screened on cellulose acetate plates (Titan III, Helena Laboratories) as described in Richardson *et al.* (1986) except that the respective enzyme stain was applied as a 1 per cent molten agar solution (J. Doran and P. Sunnucks, pers. comm.). For improved resolution, transferrin (*Tf*) was screened using vertical 12 per cent polyacrylamide gels with the buffer system of Gahne *et al.* (1977).

Identification of microsatellite loci

Polymorphic microsatellite DNA loci were identified within our laboratory or acquired from other researchers. The microsatellite loci screened are listed in Table 2.

Table 1 Soluble proteins initially screened for polymorphism in Soay sheep from the Hirta population. All proteins were screened in white cell fractions except where specified otherwise

Monomorphic loci ($n = 28$)	Animals screened	
4-Methyl umbelliferyl propionate Esterase (red cells)	40	
alpha-Naphthyl acetate esterase (red cells)	40	
Nucleoside phosphorylase	60	
Lactate dehydrogenase (2 loci)	50	
Superoxide dismutase (2 loci)	70	
Peptidase A	50	
Peptidase B	40	
Acid phosphatase-1 (red cells)	40	
Carbonic anhydrase-2	40	
Glyoxalase-1	40	
Diaphorase (NADH)-1	40	
Pyruvate kinase-1	40	
6-Phosphogluconate dehydrogenase	40	
Inorganic pyrophosphatase	40	
Malic enzyme-cytoplasmic	50	
Malate dehydrogenase-cytoplasmic	60	
Glutamate pyruvate transaminase	50	
Mannose phosphate isomerase	40	
Phosphoglucomutase (2 loci)	40	
Glucose phosphate isomerase	20	
Glucose dehydrogenase	20	
Albumin (plasma)	300	
Glutamate oxaloacetate transaminase-cytoplasmic	100	
Adenylate kinase (2 loci)	100	
Polymorphic loci ($n = 6$)		**Alleles**
Transferrin (plasma)	1168	7
Haemoglobin beta chain (red cells)	872	2
Isocitrate dehydrogenase-cytoplasmic	905	3
Glutamate oxaloacetate transaminase-mitochondrial	961	2
Adenosine deaminase	960	2
Peptidase C (variant frequency 10%, scoring unreliable)	50	2

Laboratory identification of loci Microsatellite regions were isolated from a genomic library of sheep DNA following the procedure of Rassmann *et al.* (1991) and three clones containing microsatellite repeats were identified (designated OarDB2, OarDB3 and OarDB6). Oligonucleotide primers were designed for these loci using the 'Oligo' program of Rychlik & Rhoads (1989), and synthesized locally. Primers were

similarly designed for a $(CT)_{23}$ repeat found in the upstream nontranslated region of the ovine pituitary adylate cyclase activating polypeptide mRNA (sequence previously described by Kimura *et al.*, 1990); the simple sequence region was designated *OPACAP*.

Loci acquired from other researchers Other microsatellite loci used in our study were identified elsewhere using similar procedures. Ovine microsatellite loci *MAF18, MAF35, MAF45* and *MAF65* were identified at the AgResearch Molecular Biology Unit, University of Otago, New Zealand (Crawford *et al.*, 1990; Swarbrick *et al.*, 1991, 1992; Buchanan *et al.*, 1992), and primers were donated by F. Buchanan and A. Crawford. Microsatellite locus *BOVIRBP* was derived from the downstream noncoding region of the bovine interphotoreceptor retinoid-binding protein gene (Borst *et al.*, 1989). This locus is described by Moore *et al.* (1991) but we used different primers, originally designed and provided by D. MacHugh, Department of Genetics, Trinity College, Dublin.

Microsatellite screening

DNA was isolated from ear punches or white blood cells by standard proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989).

Microsatellite loci were amplified as follows: approximately 50 ng genomic DNA was used as template for 2 pmol of each primer in a 10 μL reaction overlaid with 1 drop of mineral oil (reaction conditions: 0.1 mM dATP, dGTP and dTTP; 0.01 mM dCTP; 0.5 mM $MgCl_2$; 10 per cent dimethylsulphoxide; <1 μCi alpha-^{32}P dCTP; 1 × 'PARR' buffer (Cambio); 0.25 units *Taq* polymerase). A PREM III thermocycler (Quatro Biosystems) was used with the following 2-stage programme: an initial 94°C denaturation for 2 min was followed by 7 cycles of 94°C denaturation for 30 s, 52°C annealing for 1 min and 72°C extension for 30 s, and 25 cycles of 89°C denaturation for 30 s, 54°C annealing for 1 min and 72°C extension for 30 sec.

On completion of the amplification cycle, 8 μL of the reaction was added to 4 μL of sequencing loading buffer (95 per cent formamide, 20 mM EDTA, 0.05 per cent bromophenol blue and 0.05 per cent xylene cyanol). The reactions were denatured by heating to 75°C prior to loading 3 μL into lanes of a 50 cm standard denaturing sequencing gel (6 per cent polyacrylamide/8 M urea/TBE buffer; 'Sequagel' (National Diagnostics)). As a size marker, a nonrecombinant M13 mp18 sequencing reaction was also loaded. The amplification products were electrophoresed for approximately 2.5 h and the completed gel was fixed by soaking in 10 per cent methanol/5 per cent acetic acid for 10 min. The gel was dried under vacuum at 90°C and exposed to pre-flashed X-ray film for 8 to 12 h at room temperature, without intensifying screens.

Following an initial survey of each microsatellite locus over at least 30 sheep, we investigated which polymorphic loci could be coamplified in a single tube and resolved on the same gel ('multiplexed'). Six microsatellite 'study' loci were subsequently screened in a large sample of study sheep.

Statistical analysis

Locus variability Since genetic data were collected from several cohorts of sheep, the overall sample was examined for possible heterogeneity of genotypes between years. Data for the 11 study loci (five proteins and six microsatellites) were tabulated by genotype and cohort, and assuming a Poisson response distribution, the number of observations in each cell was analysed by generalised linear regression using GENSTAT 5 (Payne *et al.*, 1987). Since no significant heterogeneity of genotype frequency was observed between cohorts, the data were pooled and from them estimates of the allele frequencies were made using confidence limits obtained from the within-population variance of Weir (1990). Locus-specific heterozygosity (h) was estimated as the observed frequency of heterozygotes in the sample. The locus-specific sampling variance of heterozygosity $[V_{s1}(h)]$ was estimated by the formula of Nei & Roychoudhury (1974). Average heterozygosity (H) was calculated separately for protein and microsatellite loci using data from both 'study' and non-'study' loci (nonpolymorphic protein loci and nonmultiplexed microsatellite loci). The total sampling variance, $V(h)$, of the average heterozygosity per locus was calculated as the variance in h, and partitioned into two components, the interlocus $[V_y(h)]$ and intralocus $[V_s(h)]$ variances (Nei & Roychoudhury, 1974; Nei, 1987). The SE for each average was estimated as $[V(h)/m]^{1/2}$, where $V(h)$ is the total sampling variance over m loci.

Allelic disequilibrium Estimated allele frequencies were used to calculate Hardy–Weinberg equilibrium (HWE) frequencies of genotypes. These were tested against observed genotype frequencies using the G statistic approximated more closely to the chi-square distribution for small sample sizes by Williams' correction (G_{adj}; Sokal & Rohlf, 1981). Any association between alleles at the same locus would be detected by significant departures from HWE. False rejection of the null hypothesis (HWE) at an overall significance

level of α' for 11 tests was reduced by imposing a critical significance level for individual tests, α, which was estimated from Sidak's multiplicative inequality ($\alpha = 1-(1-\alpha')^{1/11}$; Weir, 1990).

Comparison of levels of variation with previous data The average protein heterozygosity and interlocus variance in heterozygosity observed in the Hirta Soay sheep were compared with data summarized by Nevo *et al.* (1984) and Fuerst *et al.* (1977). Many population studies of protein variation have sampled approximately the same sample of protein loci, and any effects from studies using different selections of loci should be minimal (Fuerst *et al.*, 1977). Microsatellite variation was compared with protein variability.

Results

Protein variation

Table 1 lists the 34 soluble proteins analysed for polymorphism in the Hirta population and the results of the survey. Five of the six polymorphic protein loci (*β-Hb, Idh-1, Got-2, Ada* and *Tf*) were adopted as 'study' loci and screened in a large sample of sheep. The sixth polymorphic locus, peptidase C (*Pep-C*) was difficult to score and was not screened further.

Microsatellite variation

Table 2 lists the nine microsatellites analysed for polymorphism in the Hirta population and the results of the survey. Multiplex trials identified two groups of three microsatellite loci (*OPACAP, BOVIRBP* and *MAF65; MAF45, MAF18* and *MAF35*) which could be multiplexed. These six microsatellite loci were adopted as 'study' loci and screened in a large sample of study individuals. By multiplexing the PCR reaction and using a 96-lane sequencing gel (Bio-Rad), up to 40 individuals could be screened for these six microsatellite loci within 24 hours.

Statistical analysis

Locus variability Allele frequencies for all study loci, pooled across all individuals are shown in Table 3. The locus-specific heterozygosity (h) and its SE for each locus are also shown in Table 3. These locus-specific heterozygosities were combined to give separate estimates of the average heterozygosity per protein and microsatellite locus of the population. These estimates included data from the other protein and microsatellite loci tested in the population as well as the 11 'study' loci, as follows.

(i) *Protein loci*; the average heterozygosity of protein loci, including 28 monomorphic loci and peptidase-C with a 10 per cent variant frequency (see Table 1), was estimated as $\hat{H}_p = 7.78$ per cent ± 3.25 per cent (SE). (ii) *Microsatellite loci*; the average microsatellite heterozygosity including OarDB2, OarDB3 and OarDB6 (where h ($\pm$SE) = 0.382 (0.060), 0.394 (0.021), 0.361 (0.014), respectively) (Table 2), was estimated as $\hat{H}_m = 50.93$ per cent ± 4.48 per cent (SE).

Allele disequilibrium Hardy–Weinberg analysis of the 11 study loci revealed one locus, microsatellite *OPACAP*, that showed significant deviation from expected genotype frequencies ($G_{adj} = 13.77$, 3 d.f., $P < 0.005$). Further investigation of this disequilibrium was undertaken by pooling the rarest allele, '*217*' (frequency = 0.0031), with the next rarest allele, '*215*' (frequency = 0.2508). This confirmed that there was a significant heterozygote excess in the population ($G_{adj} = 12.08$, 1 d.f., $P < 0.001$, $n = 646$ individuals).

Table 2 Microsatellite DNA loci screened in Soay sheep from St. Kilda

Locus	Source*	Typical repeat motif	Animals scored	Number of alleles	Allele size range (bp)
OarDB2	Cloned	$(GT)_{22}$	34	4	156–168
OarDB3	Cloned	$(GT)_{20}$	33	3	185–189
OarDB6	Cloned	$(GT)_{16}$	36	2	115–119
OPACAP	Sequence	$(CT)_{23}$	646	3	213–217
BOVIRBP	Sequence	$(CA)_8TACT(AC)_8(AT)_4$	658	3	146–156
MAF18	Cloned	$(GT)_9TA(GA)_5$	676	3	120–128
MAF35	Cloned	$(GT)_{10}$	671	4	106–114
MAF45	Cloned	$(GT)_{25}$	667	6	130–166
MAF65	Cloned	$(AC)_{20}$	654	4	124–132

*For origin, see text. The primer sequences for loci *OarDB2,3,6* and *OPACAP* will be described elsewhere and are available from the authors.

Table 3 Allele frequencies and numbers (n) of animals scored for the polymorphic study protein and microsatellite DNA loci in the Hirta population of Soay sheep. Also shown is the estimated heterozygosity ($\hat{h}$) of each study locus ± SE. The standard error was obtained from the locus-specific sampling variance of heterozygosity

Locus	Allele	Frequency	Heterozygosity $\hat{h} \pm$ SE
Tf	*A*	0.154	0.791 ± 0.003
$n = 1168$	*G*	0.287	
	B	0.054	
	C	0.014	
	D	0.171	
	M	0.277	
	E	0.043	
β-Hb	*A*	0.470	0.503 ± 0.002
$n = 872$	*B*	0.530	
Idh-1	*S*	0.672	0.515 ± 0.011
$n = 905$	*M*	0.193	
	F	0.135	
Got-2	*S*	0.808	0.315 ± 0.011
$n = 961$	*F*	0.192	
Ada	*S*	0.761	0.342 ± 0.010
$n = 960$	*F*	0.239	
OPACAP	*213*	0.746	0.427 ± 0.012
$n = 646$	*215*	0.251	
	217	0.003	
BOVIRBP	*146*	0.191	0.654 ± 0.006
$n = 658$	*152*	0.326	
	156	0.483	
MAF18	*120*	0.639	0.510 ± 0.011
$n = 676$	*122*	0.094	
	128	0.267	
MAF35	*106*	0.384	0.550 ± 0.007
$n = 671$	*108*	0.538	
	110	0.004	
	114	0.075	
MAF45	*130*	0.034	0.759 ± 0.006
$n = 667$	*148*	0.355	
	150	0.322	
	152	0.144	
	158	0.092	
	166	0.052	
MAF65	*124*	0.630	0.547 ± 0.011
$n = 654$	*126*	0.069	
	128	0.284	
	132	0.018	

Comparisons of levels of variation with previous data The average heterozygosity per protein locus for the Hirta population of Soay sheep was estimated as 7.78 per cent ± 3.25 per cent. Compared to the average protein heterozygosity of 183 other mammalian species or subspecies surveyed by Nevo *et al.* (1984), the average protein heterozygosity of Soay sheep lies within the top 17 per cent of the distribution (Fig. 1; mean mammalian heterozygosity = 4.14 ± 0.25 per cent (SE)). Contrary to *a priori* expectations (see Introduction), the level of protein variation in the Hirta population of Soay sheep appears high.

Fuerst and coworkers (1977) modelled the theoretical relationship between average protein heterozygosity and the interlocus variance in heterozygosity predicted by the infinite allele mutation–drift model. They supported this theoretical relationship by plotting the observed average heterozygosity against interlocus variance of heterozygosity for 95 vertebrate species (Fig. 2). Our figures for Hirta Soay sheep fall outside the 95 per cent confidence limits for the distribution, having relatively high interlocus variance in heterozygosity in relation to average heterozygosity (Fig. 2).

Our microsatellite data clearly show the greater variability of this class of marker compared with proteins. Out of 34 protein loci tested just six were found to be polymorphic, whereas all nine microsatellite loci tested were polymorphic. Furthermore, the six polymorphic microsatellite study loci had an average of 3.8 alleles, whereas the five polymorphic protein study loci had an average of 3.2 alleles, most of which was accounted for by a single locus, *Tf*.

This observation indicates the superiority of microsatellite over protein markers for many population genetic studies. However, this superiority is a symptom of prior selection procedures for microsatellite loci. For example, the loci we used were either known to be polymorphic in other sheep or cattle or contained sufficiently long stretches of dinucleotide repeats to have a high probability of being polymorphic. Since selection procedures of varying intensity also apply to other microsatellite data sets (e.g. Weissenbach *et al.*, 1992), it is not possible to compare levels of microsatellite variation across species at present.

Discussion

Level of protein variation

The Soay sheep population of Hirta, St. Kilda has an observed protein heterozygosity of 7.78 per cent, placing it well above the mean for mammals (4.14 ± 0.25 per cent (SE); Nevo *et al.*, 1984) and in the top 17 per cent of the distribution for mammals (Fig. 1).

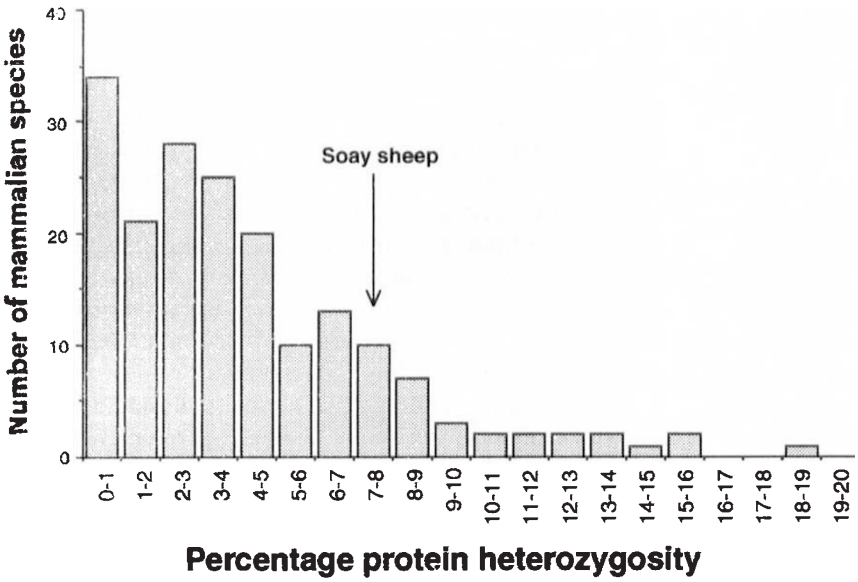

Fig. 1 Distribution of average protein heterozygosity for 183 mammalian species or subspecies (data from Nevo *et al.*, 1984). Average heterozygosity per protein locus for the Hirta population of Soay sheep was estimated at 7.78 ± 3.25 per cent.

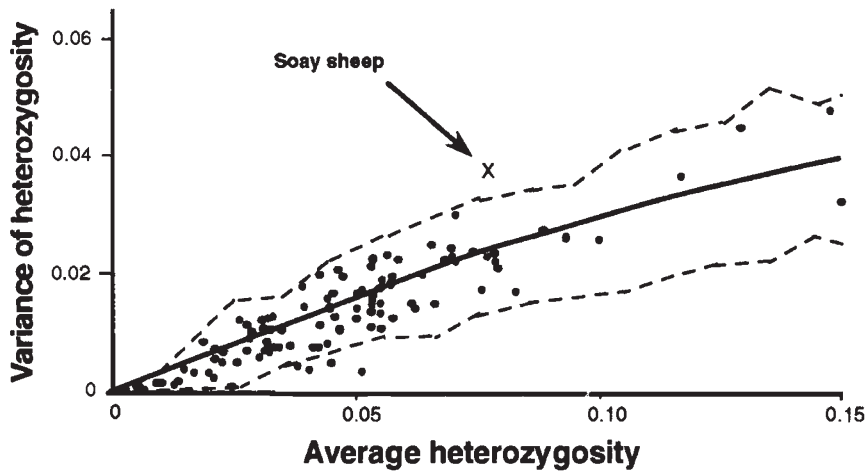

Fig. 2 Relationships (from Fuerst *et al.*, 1977) between average protein heterozygosity [$\hat{H}$] and the interlocus variance of heterozygosity [$\hat{V}_{\gamma}(h)$] for 95 vertebrate species. Protein data for the Soay sheep are added from this study. Dotted lines represent the 95 per cent confidence limits estimated by Fuerst *et al.*

Since, if anything, large mammals have lower heterozygosities than small mammals (Wooten & Smith, 1985), and since the study population has a history which would appear to promote loss of genetic variation, this result requires some explanation.

Though comparative data are limited, Hirta Soays also appear to have relatively high levels of protein variability among sheep populations. Two loci which have been well documented are *Tf* and *Hb*, at which Soays have specific heterozygosities of 79.1 per cent and 50.3, respectively. Commercial sheep breeds (with much larger census population sizes and varying degrees of isolation) have a mean *Tf* heterozygosity of 69.7 per cent (range 60.3–77.5 per cent, $n = 29$ populations) and a mean *Hb* heterozygosity of 27.6 per cent (range 0–49.5 per cent, $n = 24$ populations) (Nix *et al.*, 1969; Manwell & Baker, 1977; Weimer *et al.*, 1984; Soysal *et al.*, 1986; Clarke *et al.*, 1989b; Wang *et al.*, 1990; Zanotti Casati *et al.*, 1990; Nguyen *et al.*, 1992). Other ancient and rare British sheep breeds appear to have lower heterozygosities at *Tf* (Portland 68 per cent, Manx Loghtan 65.9 per cent and Hebridean 45 per cent) and at *Hb* (Portland 14.7 per cent, Manx Loghtan 22.6 per cent and Hebridean 12.1 per cent) (Clarke *et al.*, 1989a). This is further reflected in seven rare Dutch sheep breeds: mean heterozygosity at *Tf* 69.6 per cent (range 54.6–78.0 per cent) and *Hb* 38.3 per cent (range 9.5–48.0 per cent) (Buis & Tucker, 1983). Finally, wild sheep species appear to have considerably less variation at these same loci (Sage & Wolff, 1986; Hartl, 1990; Wang *et al.*, 1990; 1991).

Explanations for high level of protein variation

Broadly, three factors could be contributing to the high values for average protein heterozygosity and interlocus variance in heterozygosity.

Drift during successive population crashes Addressing first the issue of high interlocus variance in heterozygosity, a theoretical study by Li & Nei (1975) drew attention to the fact that in transient populations, interlocus variance in heterozygosity may actually increase before attaining its equilibrium value. On the other hand, given parameters typical of protein variation observed in natural populations, any increase in variance of heterozygosity predicted by their models was relatively small (see Table 1, Li & Nei (1975)). Typically the ratio of $V_\gamma(h)$ to H in transient populations, recently reduced in number, was similar to that of an equilibrium population (Fuerst *et al.*, 1977). Taking approximate figures for Soay sheep as an example, at equilibrium, the interlocus variance for a population of $N_e = 250$ (Bancroft, 1993) and average heterozygosity 7.78 per cent is expected to be 0.0214. From Li & Nei (1975) equation (15), we can estimate the interlocus variance for an equivalent transient population which has declined from 8.59 per cent heterozygosity to 7.78 per cent heterozygosity over 50 generations from founding (approximate time since introduction) and obtain the figure of 0.0240, only marginally higher than an equilibrium population, and still less than our observed variance of 0.0354. However, the study of Li & Nei (1975) concentrated on transience following a single reduction in census population size. It is not clear to us what the consequence of repeated changes in population size, such as those experienced by our study population, will be for interlocus variance in heterozygosity. It may well be that the long-term consequence is indeed a significantly elevated level.

Though they may elevate interlocus variance in heterozygosity, over time repeated population crashes should still have the effect of removing alleles from the population, and thus lowering average heterozygosity levels. It is thus difficult to understand the overall high levels of protein heterozygosity in Soay sheep without at least one other factor being involved.

Founding population on Soay The figures for protein variation on Hirta could be explained if the founding population introduced from Soay was itself quite exceptional in terms of both average protein heterozygosity and interlocus variance in heterozygosity. This could, perhaps, be the result of numerous successful introductions of non-Soay breeds to the island over the preceding centuries.

In some respects the idea that Soay sheep on Soay have even more protein variation than those on Hirta seems rather unlikely. First, as we have seen, it would mean this one population was or is more variable at its protein loci than any other sheep population tested. Second, this would be despite the fact that the population on Soay is much smaller than that on Hirta (averaging perhaps 200 animals and ranging up to maybe 500) and believed to be subject to the same cyclical crashes and polygynous mating system.

Under these circumstances, it is not clear to us what we would predict for contemporary protein variation among Soay sheep on Soay. Protein variability could be much greater than on Hirta because a differential that existed in 1932 is still present. On the other hand, it could be equal to or lower than that on Hirta because the smaller effective population size on Soay has eroded heterozygosity faster than on Hirta.

In any case, it is not trivial to go and sample Soay sheep on Soay for genetic variation. The sheer cliffs of the island make landing very difficult under unpredictable sea and weather conditions, and catching a reasonable sample of sheep on the steep slopes would be dangerous. Nevertheless, because it was achieved in 1932 to provide the introduction to Hirta, it must be feasible, and we intend to investigate the possibility.

Selection promoted by population crashes An alternative explanation is that the population crashes observed on Hirta (and thought to occur on Soay) are somehow involved in producing this unusual distribution of genetic variability via selection. For example, nonrandom mortality of particular genotypes during population crashes could influence the distribution of genetic variability within the population. The periodic population crashes suffered by the Hirta population seem likely to be periods of intense selection, and various scenarios seem possible based on this theme. First, if crashes are periods of intense selection, then fluctuation in selection intensity between years may maintain variation. The effect of fluctuation in selection intensity on genetic variation has been modelled by several investigators including Takahata (1981), who found that a large variance in heterozygosity, as found in Soay sheep, was a general feature of such models. Second, the models of Maruyama & Nei (1981) showed that a combination of mutation and overdominant selection was very powerful in increasing the mean heterozygosity and variance in heterozygosity as compared with neutral mutations. In general, even a very low mutation rate at overdominant loci was sufficient to explain the high levels of polymorphism in a finite population, even for loci with a large number of alleles. We have observed a heterozygote excess at the microsatellite locus *OPACAP*

which may reflect overdominant mechanisms. With the involvement of nematode and possibly other parasites in crash survival (Gulland, 1992; Gulland *et al.*, 1993) a third possibility is that genetic variation in Soay sheep is associated with changes in parasite genotypes (Hamilton, 1982). Finally, of course any combination of these factors may be occurring simultaneously within our population.

To a greater extent than the other factors discussed, we can test the suggestion that periodic crashes are influencing the distribution of genotypes in the Hirta population. Nonrandom mortality of individuals and molecular genotypes during population crashes is now being investigated in our individually-monitored population. We have at least one example of an apparently overdominant locus, since *Ada* heterozygotes survive population crashes better than the two other genotypes at this locus (Gulland *et al.*, 1993).

Microsatellite variation

Our study has demonstrated far greater genetic variability at microsatellite compared with protein markers. If microsatellite primers are available for the study species or related species, or if cloning and selection procedures yielding dinucleotide repeat sequences are in place, then the probability of finding polymorphism and the likely number of alleles per locus are far higher than for the average protein locus. This higher variability of microsatellite DNA is an obvious advantage over protein variability when applied to intrapopulation genetic studies or interpopulation studies where the same loci can be screened.

The value of microsatellite markers in comparisons of levels of genetic variation where different loci have been sampled, or across distantly related species must, however, be in some doubt. These comparisons are likely to involve different panels of loci subject to different laboratory selection criteria. This would be especially true for comparisons between species as, in general, microsatellite PCR primers do not work over large taxonomic distances (Moore *et al.*, 1991). However, it is clear that there are some microsatellites that have survived exceptional spans of evolutionary time (Hino *et al.*, 1993) and, as well as having applications in mapping studies, these may prove to be suitable loci with which to compare levels of variation between species.

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