Molecular genetic variation and individual survival during population crashes of an unmanaged ungulate population

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SUMMARY

Theoretical models of the effect of population bottlenecks on genetic variation assume that individuals are removed at random from the population. We investigated this assumption in a naturally regulated, unstable population of Soay sheep (*Ovis aries*). During rapid population declines or 'crashes', individuals were not removed at random with respect to genotype: we found associations between individual survival and certain genotypes at five polymorphic protein or microsatellite DNA loci (*Ada*, *Got*, *Tf*, MAF18 and OPACAP). Some loci appeared to show simple associations with survival whereas others had more complex interactions with crash year or age: all displayed different patterns of association between the sexes. Simple overdominance was not a general feature of our data; it seems likely that fluctuating selection, countervailing selection in different fitness components or frequency-dependent selection may explain the pattern and complexity of the associations shown at different loci. Our study cannot distinguish between selection acting at these loci or at other, closely linked loci. However, our empirical study implies that the molecular genetic outcome of population bottlenecks in natural populations does not always follow theoretical expectations based on the random removal of genotypes. Bottlenecks in which individuals are removed at random are distinct from bottlenecks in which there is scope for selection via non-random survival of individuals.

1. INTRODUCTION

During phases of drastic reduction in population size, both random and non-random survival of individuals will occur. The relative importance of these two factors is of key significance for the interpretation of molecular genetic variability within natural populations.

Despite evidence that populations generally lose genetic variability through random survival during population bottlenecks (Crow & Kimura 1979; Sing *et al.* 1973; Bonnell & Selander 1974; Nei *et al.* 1975; Wildt *et al.* 1987; Hoelzel *et al.* 1993), some populations that experience regular or irregular bottlenecks maintain high levels of genetic variation. One well documented example is the unstable population of Soay sheep (*Ovis aries*) living in the St. Kilda archipelago, Scotland (Jewell *et al.* 1974; Clutton-Brock *et al.* 1991, 1992; Grenfell *et al.* 1992). This isolated and unmanaged population has an estimated effective size (*Ne*) of around 260 (Bancroft 1993) and suffers regular population crashes in which up to 70% of individuals die. Despite these factors, the population maintains substantial genetic variation at both protein and microsatellite loci (Bancroft *et al.* 1995).

The Soay sheep example suggests that factors other than random survival during bottlenecks may help to maintain genetic variability. Indeed, there are reasons for believing that the consequences of population bottlenecks for genetic variation are not fully understood. First, experimental studies have shown that though molecular variation may be reduced in a predicted manner, quantitative variation is not and may even increase (Bryant *et al.* 1986; Polans *et al.* 1989). 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 populations (Carson 1990).

In this study we investigate whether non-random survival during population crashes in Soay sheep could contribute to the unusually high level of genetic variation. Over three successive population crashes on St Kilda, individual survival was not random with

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respect to genotype at five molecular loci. Because overdominance was not a general feature of our results, we propose alternative mechanisms by which, far from removing genetic variation, frequent population crashes may be involved in maintaining molecular variation within the Soay sheep population.

2. MATERIALS AND METHODS

(*a*) *Soay sheep on Hirta*

Soay sheep living on the island of Hirta, St Kilda, Scotland have been the focus of scientific interest since the introduction of 107 individuals (20 rams, 24 ewes, 21 ewe lambs and 22 castrated ram lambs) from the adjacent, smaller island of Soay in 1932 (Boyd 1953). Since this date the population has been isolated and free of competing herbivores, predators or management. Each summer since 1955 the total number of sheep on Hirta has been estimated by the same census method. Two periods of intensive research 1959–1968 (Jewell *et al.* 1974) and 1985 to the present (Clutton-Brock *et al.* 1991, 1992; Grenfell *et al.* 1992) have been undertaken.

The Soay sheep population varies dramatically in size, with periods of high over-winter mortality ('crashes') occurring every 3–4 years (see figure 1). Soay sheep are highly fecund, so that within one breeding season the winter carrying capacity of Hirta can be exceeded (Grenfell *et al.* 1992; Clutton-Brock *et al.* 1995). This leads to density-dependent mortality, primarily caused by starvation (Grubb 1974; Clutton-Brock *et al.* 1991) but compounded by parasitic infection (Gulland 1992; Gulland *et al.* 1993). Up to 70% of individuals die during these crashes and many more males die than females.

(*b*) *Village Bay study population*

The greatest concentration of Soay sheep on Hirta is in the Village Bay area (Jewell *et al.* 1974; Clutton-Brock *et al.* 1992). Numbers in Village Bay are correlated strongly with total numbers on Hirta (Jewell *et al.* 1974; Clutton-Brock *et al.* 1991). For more than a decade preceding 1985, a sample of Village Bay lambs ($n > 100$) were tagged and weighed at birth. Since 1985, this programme has been intensified to include all newborn lambs in the study area, which are also sampled for molecular genetic analyses. Additional body mass measurements and genetic samples have been obtained by capturing adult sheep resident in the Village Bay area each August since 1985. Together with detailed behavioural observations, these data provide comprehensive information on life history, phenotypic and genotypic traits for approximately 1650 individuals that have been alive since 1985.

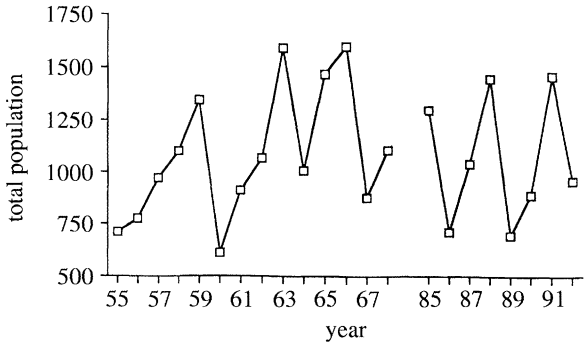

Figure 1. The cyclical population dynamics of Soay sheep on Hirta showing the annual population count of the whole island. The period 1968 to 1985 was between the two intensive studies. These unstable and apparently chaotic population dynamics have been investigated more rigourously elsewhere (Grenfell *et al.* 1992).

(*c*) *Individual survival*

When population density is low, searches for corpses are made during the lambing period (which starts in late March). Generally, few corpses are found: typically, less than 10% of the prewinter population. When population density is high and a crash is expected, daily mortality searches are made from mid-January until mid-May. Particular care is made to search the numerous stone structures or 'cleits', in which sheep shelter and die (Stevenson 1994). More than 80% of Village Bay sheep that disappear are found dead (Clutton-Brock *et al.* 1991). Those animals not seen in study area censuses during the spring and summer following a crash, and not seen in other parts of the island, are presumed dead.

(*d*) *Genotypic screening*

Tissue samples are taken whenever an animal is handled. Approximately 10 ml jugular blood (2 ml from newborn lambs) is collected in heparinized tubes and 5 mm diameter ear punches are taken before ear tagging. Whole blood is fractioned into plasma, white and red cells as described by Bancroft *et al.* (1995). Blood fractions and ear punches are stored at −20 °C on Hirta and transported to Cambridge still frozen.

Samples taken from the Hirta study population are routinely screened for five polymorphic protein and six polymorphic microsatellite DNA loci. Details of our initial survey for polymorphism and the screening methods employed are contained in Bancroft (1993), Bancroft *et al.* (1995) & Gulland *et al.* (1993). Table 1 lists the five protein and six microsatellite loci screened in a large-scale survey of the Hirta population.

(*e*) *Analysis of survival*

The analysis of survival was undertaken using logistic regression analysis (Cox 1970). Crash survival of individual sheep was considered as a binomially distributed response variable (survival = 1, death = 0). Logistic regression techniques have significant advantages over alternative methods such as contingency table analysis because factors other than the one under investigation can be controlled, including metric covariates. This is particularly valuable in the case of the Soay sheep study, as phenotypic variables such as body mass strongly affect the probability that

Table 1. *Protein and microsatellite loci screened in a large scale population survey of Soay sheep on Hirta, St Kilda*

locus	alleles	individuals scored
transferrin (*Tf*)	7	1577
β-haemoglobin (*β-Hb*)	2	1381
isocitrate dehydrogenase-cytoplasmic (*Idh-1*)	3	1376
adenosine deaminase (*Ada*)	2	1415
glutamate oxaloacetate transaminase-mitochondrial (*Got-2*)	2	1417
OPACAP[a]	3	933
BOVIRBP[b]	3	938
MAF18[c]	3	957
MAF35[d]	4	892
MAF45[e]	6	922
MAF65[f]	4	936

[a] OPACAP = DNA sequence of ovine pituitary adylate cyclase activating protein gene (published by Kimura *et al.* 1990), primers for $(CT)_{23}$ upstream non-coding repeat were designed in our laboratory.

[b] BOVIRBP = DNA sequence of bovine interphotoreceptor retinoid-binding protein gene (published by Liou *et al.* 1986; Borst *et al.* 1989). Primers were designed and donated by D. MacHugh (Department of Genetics, Trinity College Dublin).

[c–f] MAF18–MAF65 = randomly cloned ovine microsatellites (Crawford *et al.* 1990; Swarbrick *et al.* 1991, 1992; Buchanan *et al.* 1992), and primers were donated by the authors.

a sheep survives a crash (Clutton-Brock *et al.* 1992). Dependent variables (such as mass, age and genotype) were modelled as single effects, as single effects in combination or as interactions until models that accounted for the maximal amount of variation in survival were obtained. The analysis was done using the generalized linear modelling procedures of GENSTAT 5 (Payne *et al.* 1987).

Terms were included or dropped from the developing model according to the change in deviance (equivalent to the sum of squares) explained by the term in question, which was distributed approximately as chi-square. Only those terms with a significance of $p < 0.05$ were included in logistic models. This statistical approach has been applied successfully to investigate phenotypic and genetic effects on fitness components in Soay sheep (Clutton-Brock *et al.* 1992; Gulland *et al.* 1993) and red deer *Cervus elaphus* (Clutton-Brock *et al.* 1987, 1989; Pemberton *et al.* 1988; Pemberton *et al.* 1991).

We investigated survival of sheep over three population crashes occurring in the winters of 1985/86, 1988/89 and 1991/92. At each crash, our study population can be divided into subpopulations that have previously experienced zero, one, two, three or more than three crashes. This has two consequences for analysis: first, as surviving one crash increases an animal's probability of surviving subsequent crashes (until the individual senesces), phenotypic or genotypic traits conferring survival may become enriched in older cohorts; second, even if no such enrichment occurs, because traits such as the sex and genotype of an animal do not change, if the same animals are considered for more than one population crash, these data points will be non-independent between crashes. There were various possible ways around these difficulties; for this analysis we chose the most conservative and considered only 'crash-naive' animals. This group consisted of individuals born in 1989 1990 and 1991 entering the 1991/92 crash; individuals born in 1986 1987 and 1988 entering the 1988/89 crash; and individuals born in 1983 1984 and 1985 entering the 1985/86 crash. (There is strong evidence that a crash also occurred in 1981/82.) Selection of 'crash-naive' animals therefore restricted the analysis to animals of two years and younger. However, younger animals have the greatest mortality rate (Grubb 1974; Clutton-Brock *et al.* 1992) and might be expected to experience stronger selection pressure than older sheep. Preliminary investigations show that some of the mortality patterns reported here may be similar to those for older animals during population crashes.

Logistic models were constructed separately for each sex because crash survival is strongly sex-biased. Many males die during a crash because their large energy expenditure during the November rut presumably depletes their fat reserves (Stevenson 1994). We were interested in contrasting mortality patterns between the sexes within our survival models without allowing the models to become too large because of the addition of many sex-interaction terms.

The variables considered in logistic models of crash survival are listed in table 2. For each model, we attempted to fit a number of phenotypic and environmental variables (crash-year, mass, age, treatment, coat colour and horn type) expected to be associated with survival. These terms were fitted as single effects, as single effects in combination and as interactions before attempting to fit terms corresponding to the various molecular genotypes.

The protein and microsatellite loci had different numbers of genotypes and it was difficult to adopt any general rule for the classification of molecular data. For most loci, we considered the maximum number of genotypic classes containing appropriate sample sizes for a locus and then grouped classes that appeared to have similar behaviour with respect to survival.

For the phenotypic and environmental variables, and protein genotypes, maximal models covering all three crashes were developed for each sex, including any terms that significantly ($p < 0.05$) reduced the residual deviance (model I for rams, model II for ewes).

Microsatellite genotypes were obtained primarily for paternity analysis of recent cohorts (Bancroft 1993). Only the last two crashes (1988/89 and 1991/92) could be analysed with respect to microsatellite genotype. Therefore, two further models, including microsatellite genotypes, were developed for each sex by restricting the analyses to these later two crashes (model III for rams, and IV for ewes).

(*f*) *Presentation of survival analysis*

In the results, each final model is described in a

Table 2. *Variables fitted to the binomially distributed response variable (survival = 1, death = 0) in sex-specific models of mortality*

phenotypic and environmental variables	type	details
mass	continuous	body mass at capture in preceding August
age	factorial	classed as lambs, yearlings and two-year-olds
treatment	factorial	'1' if treated. Other '0'[a]
coat colour	factorial	dark–wild, light–wild, dark–self, light–self[b]
horn type	factorial	horned, scurred, polled and broken[b]
crash year	factorial	fitted as simple term and as interactions (1985/86, 1988/89, 1991/92)
molecular genotypes		
protein genotype (*Tf*, *Hb*, *Idh*, *Got* and *Ada*)	factorial	each locus classified by genotype and considered as an independent factor[b]
microsatellite genotype (OPACAP, BOVIRBP, MAF18, MAF35, MAF45, MAF65)	factorial	each locus classified by genotype and considered as an independent factor[b]

[a] For purposes of other investigations, some animals were treated with anthelmintic boluses or excluded from reproduction by hormone treatment, both of which potentially increased crash survival.
[b] Some classes were pooled, details in results.

subsection and by a table. Relationships found with molecularly determined genotypes are illustrated by figures. Because raw data points fall only at survival = 1 or survival = 0 (death), graphical results of significant terms from the models are displayed using fitted values only. Fitted values were calculated for each term by averaging over the effects of all other factors and are shown plotted against the continuous variable mass or as a histogram classified by age. In general, we do not discuss associations found between phenotypic and environmental variables (such as mass and crash year) and survival in any detail as these are the main subject of other reports (Moorcroft 1991; Illius *et al.* 1994). These variables have been shown to affect survival and our analyses control for these effects.

(*g*) *Changes in allele frequency*

Loci that showed survival differences between genotypes were investigated for evidence of a resulting change in allele frequency as follows. Allele counts were tabulated by allele and survival and loci that showed significant crash year or age interactions in the logistic regression analysis were additionally tabulated by crash year or age. The observed number of alleles in each cell was then analysed by generalized linear regression, assuming a Poisson response distribution. This approach was equivalent to contingency table analysis but enabled the models to be extended easily to investigate allele frequency changes at the loci which, from logistic analysis of crash survival, appeared to show different survival effects in different crashes or ages. Significant changes in allele frequency were investigated by comparing the deviance explained by the relevant interaction term against the chi-square distribution.

3. RESULTS

(*a*) *Male survival and protein genotypes*

(i) *Phenotypic and environmental variables*

The probability of survival in males varied with age, mass, treatment and crash year. In addition, both mass and treatment interacted with crash year. The changes in deviance due to these variables, and associated p values, are shown in table 3. Crash year and its interactions are important in the model because the crashes monitored varied in intensity (1991/1992 was less severe than the other two crashes, see figure 1).

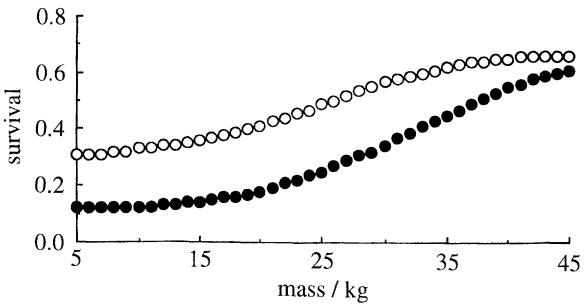

Figure 2. Differential crash survival of transferrin type in males ($G = 15.13$, 1 d.f., $p < 0.001$; model I). The probability of survival for the *Tf* types is shown by the fitted curves (open circles, *Tf* homozygotes, $n = 48$; filled circles, *Tf* heterozygotes, $n = 199$). Fitted values were calculated by averaging over the effects of all other factors.

(ii) *Protein genotypes*

Transferrin (Tf) genotype

At the *Tf* locus (seven alleles), 27 of the 28 possible genotypes have been found in the study population, many at frequencies too low for statistical treatment. However, if genotypes were pooled into 'homozygote' and 'heterozygote' classes, homozygotes had significantly higher survival ($G = 15.13$, 1 d.f., $p < 0.001$). For example, a male of 20 kg had a probability of survival of 0.4 if homozygous at the *Tf* locus, compared to only 0.2 for a similarly sized heterozygote male (see figure 2).

The homozygous class at *Tf* consists mainly of homozygotes for the two most common alleles ('GG' and 'MM'). A highly significant association remained if male survival was analysed with respect to *Tf*

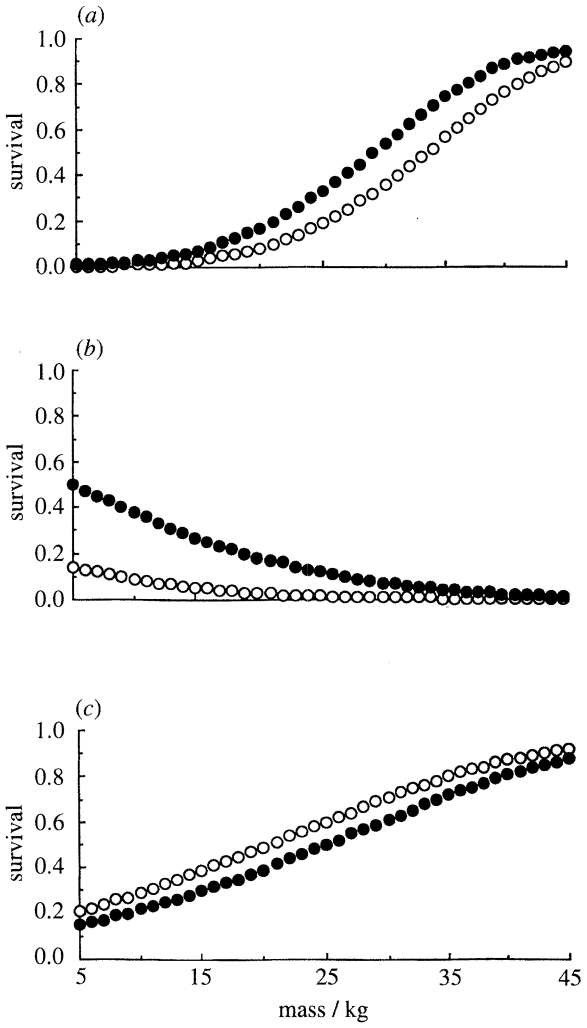

Figure 3. Differential crash survival of *Ada* genotypes in males (*Ada*: $G = 0.57$, 1 d.f., $p > 0.1$; *Ada*–crash year: $G = 7.64$, 2 d.f., $p < 0.05$; model I) during different crashes. (*a*) 1985/86: open circles, *Ada* homozygotes, $n = 56$; filled circles, *Ada* heterozygotes, $n = 24$; (*b*) 1988/89: open circles, *Ada* homozygotes, $n = 50$; filled circles, *Ada* heterozygotes, $n = 29$; (*c*) 1991/92: open circles, *Ada* homozygotes, $n = 49$; filled circles, *Ada* heterozygotes, $n = 39$.

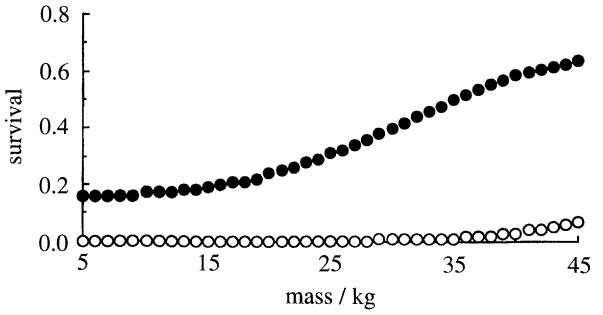

Figure 4. Differential crash survival of *Got* genotypes in males ($G = 5.33$, 1 d.f., $p < 0.05$; model I). Open circles, *Got* 'FF', $n = 8$; filled circles, Got 'other genotypes', $n = 239$.

Table 3. *Analysis of deviance table for the logistic regression of crash-naive males over three crashes, including protein genotypes*

term	d.f.	deviance	*p*
mass	1	3.93	< 0.050
crash year	2	57.41	< 0.001
mass–crash-year interaction	2	8.17	< 0.025
age	2	10.52	< 0.010
treatment	1	18.47	< 0.001
treatment–crash year interaction	1	4.49	< 0.050
Tf (homozygotes/ heterozygotes)	1	15.13	< 0.001
Ada (homozygotes/ heterozygotes)	1	0.57	NS
Ada–crash year interaction	2	7.64	< 0.025
Got ('FF'/'other genotypes')	1	5.33	< 0.025
residual	232	146.70	
total	246	278.36	
genetic terms (inc interactions)	5	28.67	
other terms	9	102.99	

genotypes grouped into 'GG', 'MM' and 'other genotypes'. Homozygotes 'GG' and 'MM' had a significantly higher probability of crash survival than the combined class of all other genotypes ($G = 11.08$, 2 d.f., $p < 0.01$).

To demonstrate that the association of *Tf* genotype and survival was indeed a difference between homozygotes and heterozygotes, two additional models were investigated using different groupings of the *Tf* genotypes: (i) the survival of 'GG' homozygotes was significantly greater than 'G' heterozygotes ($G = 8.12$, 1 d.f., $p < 0.01$, $n = 126$); and (ii) the survival of 'MM' homozygotes was significantly greater than the 'M' heterozygotes ($G = 4.14$, 1 d.f., $p < 0.05$, $n = 121$).

Adenosine deaminase (Ada) genotype

Initial investigations by Moorcroft (1991) and later by Gulland *et al.* (1993) suggested that the 'SF' genotype at the diallelic *Ada* locus showed higher survival. After these investigations, *Ada* genotypes were classified into 'homozygotes' and 'heterozygotes'. In this analysis of crash-naive individuals, the average result of *Ada* genotype over the three crashes was insignificant ($G = 0.57$, 1 d.f., $p > 0.1$), however, a significant *Ada*–crash-year interaction was present ($G = 7.64$, 2 d.f., $p < 005$). Heterozygotes showed increased probability of survival in the 1985/86 crash and the 1988/89 crash. In the latter, a 20 kg ram only had 0.03 probability of survival if homozygous for *Ada*, compared to 0.25 if heterozygous. During the less severe crash of 1991/92, heterozygotes appear to have a slightly reduced survival probability compared to homozygotes (see figure 3*a–c*).

Glutamate oxaloacetate transaminase-mitochondrial (Got) genotype

There was a significant association between genotype at *Got* and crash survival of rams. Rams homozygous for the 'F' allele had a negligible probability of survival compared to other genotypes ($G = 5.33$, 1 d.f., $p < 0.05$; see figure 4). However, the significance of this term is based on a small number of rams with the rare *Got* 'FF' genotype ($n = 8$, all of which died).

Table 4. *Analysis of deviance table for the logistic regression of crash-naive females over three crashes, no significant effects of protein genotypes*

term	d.f.	deviance	p
mass	1	51.06	< 0.001
crash year	2	31.32	< 0.001
treatment	1	6.31	< 0.025
coat colour ('dark–wild'/ 'other coat types')	1	3.38	NS
coat–crash year interaction	2	9.59	< 0.010
horn type ('scurred'/ 'other horn types')	1	0.79	NS
horn type–crash year interaction	2	7.63	< 0.0025
residual	272	278.39	
total	282	388.47	

Table 5. *Analysis of deviance table for the logistic regression of crash-naive males over two crashes, considering protein, microsatellite and other terms*

term	d.f.	deviance	p
crash year	1	43.57	< 0.001
age	2	25.59	< 0.001
treatment	1	10.33	< 0.005
treatment–crash year interaction	1	13.62	< 0.001
Tf (homozygotes/ heterozygotes)	1	5.83	< 0.025
MAF18 ('128 containing'/ 'other genotypes')	1	4.10	< 0.050
MAF18–crash year interaction	1	4.03	< 0.050
residual	270	223.57	
total	278	330.64	
genetic terms (inc. interactions)	3	13.95	

(iii) *Maximal model I*

The three polymorphic proteins and the phenotypic and environmental variables observed above, all independently contributed to the probability of survival and were included in maximal model I. *Ada* was the only locus to show different associations in different crash years. No significant differences in survival were observed between genotypes at the two other polymorphic proteins *Idh* and *Hb*. This model accounted for approximately 44% of the total deviance in male crash survival, of which about 8% was accounted for by genetic differences (see table 3). The genetic associations each accounted for an independent source of deviance and there were no significant interaction terms between the three loci involved.

(*b*) *Female survival and protein genotypes*

(i) *Phenotypic and environmental variables*

We detected significant associations between survival, mass, treatment, and crash year (see table 4). No significant association with age was observed if mass was fitted (mass and age are highly correlated). Following initial studies by Mason (1986) and Moorcroft (1991), we also detected associations between survival and the visible genetic polymorphisms for coat colour and horn type, both of which were only significant when incorporated as an interaction term with crash year (see table 4). In brief, coat colour was important in the first two crashes, but not in the last, less severe one, while horn type was important in the first crash but not the second two crashes.

(ii) *Protein genotypes*

No significant differences were found in the survival of different genotypes at protein loci among ewes.

(iii) *Maximal model II*

This model accounted for approximately 23% of mean deviance in female crash survival but incorporated no independent effects of protein genotype (see table 4). This observation was not due to small sample sizes. Of the 283 females considered for model II, over 95% of the possible 1415 protein genotypes had been screened.

(*c*) *Male survival and microsatellite genotypes*

(i) *Phenotypic, environmental and protein genotypes*

We found significant relationships between crash survival and age, treatment and crash year and a treatment–crash year interaction (see table 5). Results for this two-crash model were therefore broadly similar to that for the three-crash model (model I) except that we could no longer show independent effects of age and mass. Fitting age, as shown in table 5, allowed us to expand our sample size for analysis from 247 to 279 even though only two crashes were being considered in this analysis as we could include animals we had failed to catch and weigh the preceding summer. As in model I, rams homozygous at the *Tf* locus had a significantly greater probability of survival ($G = 5.83$, 1 d.f., $p < 0.02$).

(ii) *Microsatellite genotypes*

MAF18 microsatellite genotype

Rams of different genotypes at the triallelic MAF18 microsatellite locus had different probabilities of survival but this altered between crashes. Individuals with the MAF18 '128' allele had a significantly greater survival probability than other genotypes during the severe crash of 1988/89 (MAF18: $G = 4.10$, 1 d.f., $p < 0.05$. MAF18–crash year interaction: $G = 4.03$, 1 d.f., $p < 0.05$). For example, a yearling ram with a genotype containing a '128' allele at MAF18 had a survival probability of 0.35 during the 1988/89 crash compared to other genotypes with a survival probability of 0.10.

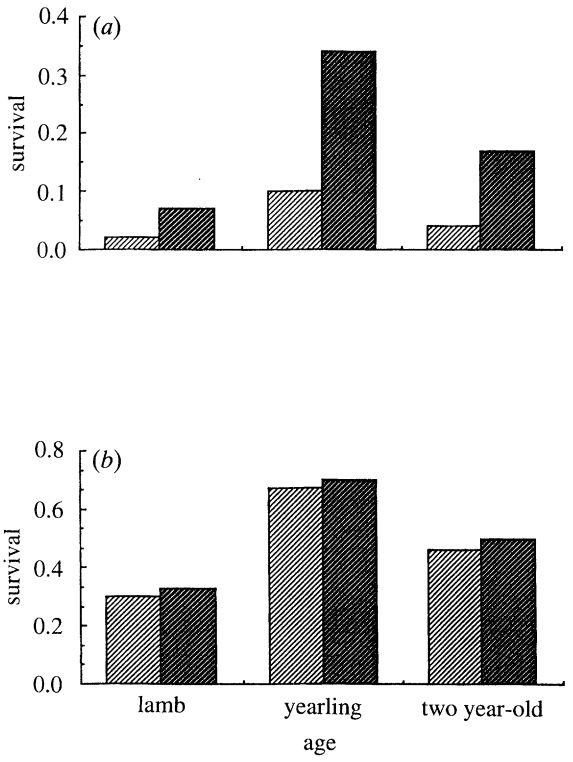

Figure 5. Differential crash survival of MAF18 genotypes in males (MAF18: $G = 4.10$, 1 d.f., $p < 0.05$; MAF18–crash year: $G = 4.03$, 1 d.f., $p < 0.05$; model III) during two population crashes. (*a*) 1988/89: dark-shaded bars, MAF18 '128 containing', $n = 54$; light-shaded bars, MAF18 'other genotypes', $n = 90$; (*b*) 1991/92: dark-shaded bars, MAF18 '128 containing', $n = 68$; light-shaded bars, MAF18 'other genotypes', $n = 67$. The terms age and mass were no longer independent in this model and hence survival probability is displayed as a histogram for the three different age classes. As mass was no longer included in this model, animals that we failed to catch and weigh the preceding summer could be included.

There was little appreciable difference between the survival probabilities of MAF18 genotypes during the less severe crash of 1991/92 (see figure 5*a*, *b*).

(iii) *Maximal model III*

No significant differences in survival were accounted for by genotypes of other protein or microsatellite loci. In particular, we could no longer find any association between survival and either *Ada* or *Got* genotype, presumably because our model no longer included the severe crash of 1985/1986. All terms described above were included into maximal model III. This model accounted for approximately 30% of mean deviance in male survival during the 1988/89 and 1991/92 crashes (see table 5), of which approximately 3.2% is accounted for by genetic (protein and microsatellite) differences.

(*d*) *Female survival and microsatellite genotypes*

(i) *Phenotypic, environmental and protein genotypes*

As in the three-crash model for females (model II), we found that mass, treatment and crash year were important in predicting crash survival in the two-crash model (see table 6). In contrast, however, we were unable to show significant associations with coat colour or horn type (see table 6), presumably because we were no longer considering the severe crash of 1985/1986 in which these variables were important.

Table 6. *Analysis of deviance table for the logistic regression of crash-naive females over two crashes, considering protein, microsatellite and other terms*

term	d.f.	deviance	p
crash year	1	38.44	< 0.001
mass	1	19.57	< 0.001
treatment	1	5.13	< 0.025
age	2	1.30	NS
OPACAP ('215 containing'/ 'other genotypes')	1	2.07	NS
OPACAP–age interaction	2	12.70	< 0.005
residual	189	193.25	
total	197	272.46	
genetic terms (inc. interactions)	5	16.065	

(ii) *Microsatellite genotypes*

OPACAP microsatellite genotype

A complicated association with survival was found for genotypes at the triallelic OPACAP microsatellite locus. After earlier work by Bancroft (1993), the OPACAP genotypes were classified into a first class for those containing a '215' allele and a second class for all other genotypes. The average effect of OPACAP genotypes was non-significant ($G = 2.07$, 1 d.f., $p > 0.1$). However, there was a significant Age–OPACAP interaction: the effect of OPACAP genotype differed between different age classes of ewe ($G = 12.70$, 2 d.f., $p < 0.01$). Therefore, the combined term including the non-significant age and OPACAP terms was significant ($G = 16.07$, 5 d.f., $p < 0.01$). There was little appreciable difference between survival of lambs or yearlings with different OPACAP genotypes, but two-year-old ewes carrying a '215' allele were considerably more likely to survive than other genotypes (see figure 6*a–c*). Indeed, all 13 two-year-old ewes with a '215' allele at OPACAP survived, while only three of the 12 ewes of other OPACAP genotypes survived.

(iii) *Maximal model IV*

All terms described above were included into maximal model IV. This model accounted for 26% of mean deviance in female survival during the 1988/89 and 1991/92 crashes (see table 6), of which 3.4% was accounted for by genetic differences. No significant differences in survival were observed for genotypes at other microsatellite or protein loci.

(*e*) *Changes in allele frequency*

Each locus that showed survival differences between genotypes was investigated for changes in allele frequency between postcrash (survived) and dead animals. No evidence for a change in allele frequency was observed at any locus, apart from the microsatellite locus OPACAP in ewes, which showed a change in

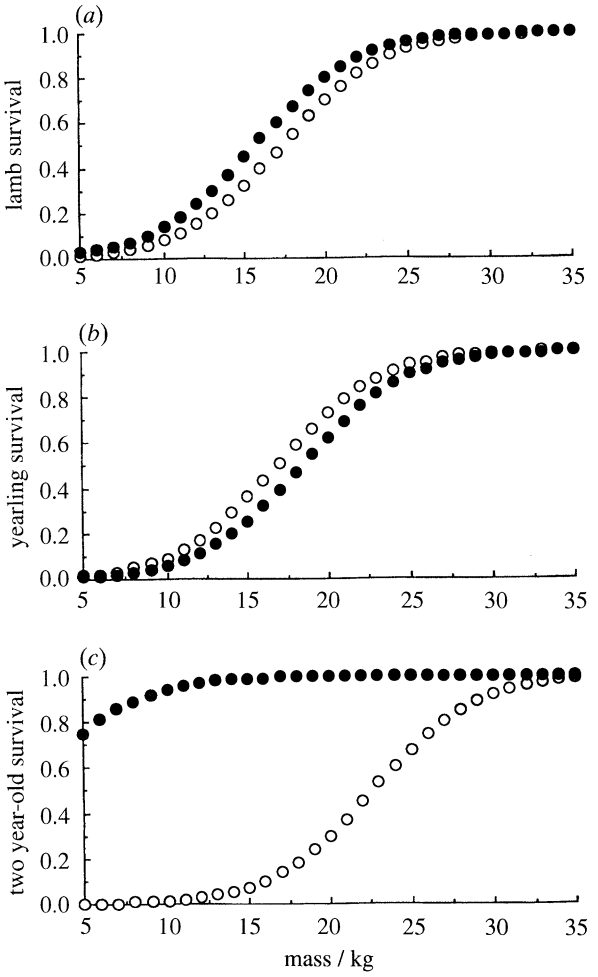

Figure 6. Differential crash survival of OPACAP genotypes in ewes for different age classes (OPACAP: $G = 2.07$, 1 d.f., $p > 1$; OPACAP–age: $G = 12.70$, 2 d.f., $p < 0.01$; model IV). (*a*) lambs: filled circles, OPACAP '215 containing', $n = 58$; open circles, OPACAP 'other genotypes', $n = 52$; (*b*) yearlings: filled circles, OPACAP '215 containing', $n = 33$; open circles, OPACAP 'other genotypes', $n = 30$; (*c*) two-year-olds: filled circles OPACAP '215 containing', $n = 12$; open circles, OPACAP 'other genotypes', $n = 13$.

Table 7. *Significant difference between allele frequencies of post-crash and dead ewes at the OPACAP locus*

(Evidence for a directional change in allele frequency towards '215' allele ('21X' the pooled class of '213' and '217'). Data were combined for the 1988/89 and 1991/92 crashes. Log linear model fitted: allele + survival + age + survival.age + allele.age. The residual deviance, was partitioned into the allele.survival ($G = 4.17$, 1 d.f., $p < 0.05$) and allele.survival.age interactions ($G = 7.42$, 2 d.f., $p < 0.025$). Note the sample size is larger than in the logistic models since this analysis could include animals which lack one or more items of individual-specific data necessary for the logistic models.)

OPACAP allele	age	died	survived
'21X'	lamb	148 (146.1)	58 (59.9)
	yearling	51 (48.8)	82 (84.2)
	two-year	22 (16.3)	27 (32.7)
'215'	lamb	52 (53.9)	24 (22.1)
	yearling	15 (17.2)	32 (29.8)
	two-year	2 (7.7)	21 (15.5)

allele frequency favouring the '215' allele, particularly for older ewes (see table 7). This observation supported the approach of pooling the small '215/215' genotypic class with the '213/215' class for the logistic model of ewe crash survival.

4. DISCUSSION

(*a*) *Associations between genotypes and crash survival in Soay sheep*

The aim of our study was to investigate whether during population crashes of Soay sheep on Hirta, individuals were removed at random with respect to molecular genotype. Here, we have described several significant associations between molecular genotypes and survival of individual sheep during these periods of drastic reduction in population size. Some loci appear as simple terms in the logistic regression models, whereas others show more complex interactions with crash year or age, and all show different patterns of association between the sexes. Our empirical study implies that the molecular genetic outcome of population bottlenecks does not always follow theoretical expectations based on the random survival of genotypes.

(*b*) *Success in detecting associations*

These results pose the question why did we observe so many locus-specific associations in this study? There are three kinds of explanations for our success in detecting associations between genotype and survival.

1. Intense selection: high mortality during population crashes is a factor specific to the Hirta population and may be sufficient to select for subtle genotypic differences in fitness. We observed more genotypic differences in survival in rams, which supports this hypothesis, because they generally suffer greater mortality than ewes.

2. The founder effect and relatively small size of the population may mean that substantial linkage disequilibrium is present in the population (this factor is also specific to the Hirta population). Each polymorphic locus may then act as a marker for a relatively large region of the genome, increasing the likelihood that it behaves as if tightly linked to a locus controlling an important fitness trait. We cannot distinguish whether evolutionary mechanisms are acting at our study loci or at other linked loci controlling fitness traits. Indeed, we observed significant associations with survival at a microsatellite tightly linked to an exon (OPACAP), a randomly cloned microsatellite (MAF18) and three proteins (*Tf*, *Ada*, *Got*). On this evidence there is no obvious difference in the behaviour of microsatellites and expressed protein loci.

3. An alternative explanation for the number and complexity of the observed genotypic associations is that it is a function of the quality of data available. Without detailed individual-specific data, including life–history, sex, phenotype and genotype, all of which potentially influence survival, these genotypic associations with crash survival may not be revealed. This hypothesis is supported by a parallel study in red deer which, using a similar investigative approach, found

associations with fitness for three protein loci (Pemberton *et al.* 1988; Pemberton *et al.* 1991). Studies that cannot control for important phenotypic factors have less chance of revealing any subtle or complex associations between genotype and fitness.

(*c*) *Level of genetic variation in Soay sheep*

Elsewhere we describe our initial investigation of protein and microsatellite DNA variability in Soay sheep (Bancroft *et al.* 1995). Extensive molecular variation was revealed at both protein loci (mean heterozygosity 7.78%) and microsatellite loci (mean heterozygosity 50.93%). 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 with other mammals, Soay sheep lie within the top 17% of the distribution of average protein heterozygosities and although comparative data is limited, Hirta Soays also appear to have relatively high levels of protein variability compared with other sheep populations. Finally, we considered a theoretical study by Li & Nei (1975) which modelled the behaviour of genetic variability in a transient population, taking into account an estimate for the effective population size based on the history and dynamics of the Hirta population. This model suggested that the average protein heterozygosity and interlocus variance observed in the Hirta Soays were greater than that predicted by mutation-drift alone: we speculated that population crashes might be involved in maintaining, rather than losing, variation (Bancroft *et al.* 1995). Although we found little evidence of changes in allele frequency, it is an unexpected feature of the results described in this paper that most of the significant relationships found between genotype and survival would appear to actually promote loss of genetic variation; namely, increased survival of homozygotes or genotypes carrying particular alleles. In this section we discuss these observations in the light of four mechanisms which could maintain genetic variation.

First, overdominance is the expected outcome of Fisher's (1930) theory of the evolution of dominance and will maintain a balanced polymorphism at a locus with a relative advantage of heterozygotes over homozygotes (Greaves & Ayers 1969; Pogson 1991). In the analysis of Soays shown here, only the protein locus *Ada* appears to show simple heterozygote advantage (in males) over population crashes.

Second, fluctuating selection between years may maintain variation in the Hirta population of Soay sheep. The effect on genetic variation of fluctuating selection intensity and direction around a long-term mean of zero has been modelled by several investigators who focused on the rate at which individual alleles were lost in such systems (e.g. Kimura 1954; Gillespie 1981). Takahata (1981) investigated changes in single locus variability by modelling the effect of fluctuating selection caused by temporal and spatial heterogeneity of environments. A large variance in heterozygosity, as found in Soay sheep (Bancroft *et al.* 1995), was a general feature of such models.

Third, countervailing selection (antagonistic pleiotropy) for alternative genotypes in other fitness components could maintain polymorphism (Rose 1982). This could act either through exact balance in the fitness of homozygous genotypes, or through 'marginal overdominance' (Wallace 1968) in which heterozygotes have the highest average fitness (Mueller *et al.* 1985; Vrijenhoek *et al.* 1992). All the genotypic associations with crash survival described in the results, apart from that involving *Ada*, appear to favour one or more alleles rather than heterozygote versus other genotypes. However, our analysis has only investigated one fitness component (crash survival). We have not yet investigated individual variation in fitness components such as female fecundity, offspring rearing success and male mating success in relation to genotype in Soay sheep. However, Pemberton *et al.* (1991) showed opposing fitness payoffs for protein genotypes in juvenile mortality and female reproductive traits in female red deer and suggested this could maintain two protein polymorphisms in the population. In this context, there are also particular reasons for exploring Soay sheep transferrin, since countervailing selection has been implicated in the maintenance of *Tf* polymorphisms in captive rhesus monkeys, *Macaca mulatta* (Smith & Small 1982), whereas Ashton (1965) reported fertility differences between *Tf* genotypes in cattle.

Finally, frequency-dependent processes could be important for maintaining variation in Soay sheep. For example, interactions between host and parasite would lead to an evolutionary arms race at loci controlling for resistance and virulence, producing no stable or equilibrium allele frequencies yet maintaining genetic variability (Hamilton 1982; Barrett 1988; Seger 1988). Associations between parasite resistance and genotype have been reported for some of the protein loci considered in our study (e.g. Frelinger 1972; Altaif & Dargie 1978; Yu *et al.* 1992). The Soay sheep of Hirta suffer a substantial parasitological threat from gastrointestinal nematodes that contributes to mortality during population crashes and we have already shown that *Ada* heterozygotes in the Hirta population are associated with lower faecal egg counts (a measure of helminth infection level) at certain times of the year (Gulland 1991, 1992; Gulland *et al.* 1993). In this study, only recent cohorts of sheep and recent crashes have been investigated and we observed little evidence of changes in allele frequency. However, it is possible that over longer timescales, further significant changes in allele frequency would be revealed.

(*d*) *Levels of genetic variation in natural populations*

Although some other studies have reported the maintenance of genetic variation by heterozygote advantage (Greaves & Ayers 1969; Pogson 1991), the majority of studies do not fit this expectation (for review, see Allendorf & Leary 1986). It is clear that many evolutionary processes must influence the distribution of molecular variation within natural populations. This study has revealed many interactions

between genotype and fitness, reflecting the possible involvement of several different evolutionary processes. These complex patterns of associations not only appear to vary from locus to locus, but also at the same locus, because many locus-specific patterns of association appear to differ between crashes, sexes and ages. It is, therefore, perhaps unsurprising that single mechanisms, for example overdominance, do not adequately explain all observed data from natural populations (Allendorf & Leary 1986). Several processes appear to affect molecular variation in the Hirta population of Soay sheep, and if suitable individual-specific data were available, it is possible that similar observations would be made in other natural populations.

Our study shows that the molecular-genetic outcome of population declines in an unmanaged sheep population does not necessarily follow theoretical or experimental expectations based on the random removal of genotypes. Therefore, Wright's (1931) expression relating effective population size to loss of heterozygosity may not be universally applicable to environmentally induced declines in population number. As this has ramifications in many areas of evolutionary biology, we suggest further investigation is required into the behaviour of genetic variation during population declines caused by environmental change.

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