

ST. KILDA SOAY SHEEP PROJECT: ANNUAL REPORT 2003

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POPULATION OVERVIEW

The sheep population on Hirta entered 2003 at a relatively low level and, as a result, there was little mortality in the first few months of the year. Only four sheep were found dead within the study area between January and May of 2003. Lambing recruitment began on the 26th of March with 90% of lambs born surviving (Fig.1).

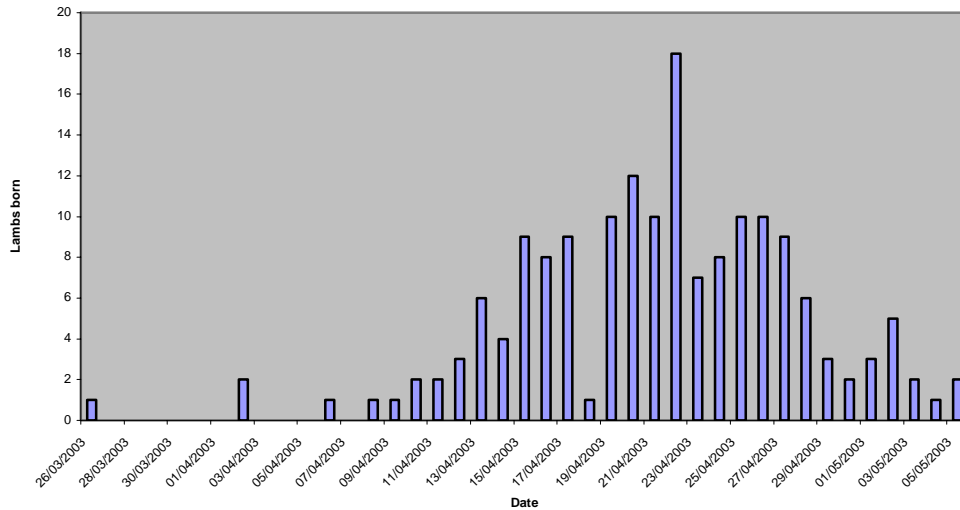


Figure 1. The temporal distribution of lamb births during 2003.

In December 2003, 612 tagged sheep were believed to be alive on Hirta, of which 496 regularly used the study area, a total increase of nearly 48% on the previous year. The age distribution of the population is shown in Fig. 2 and changes in sheep numbers in the study area over time are shown in Fig. 3.

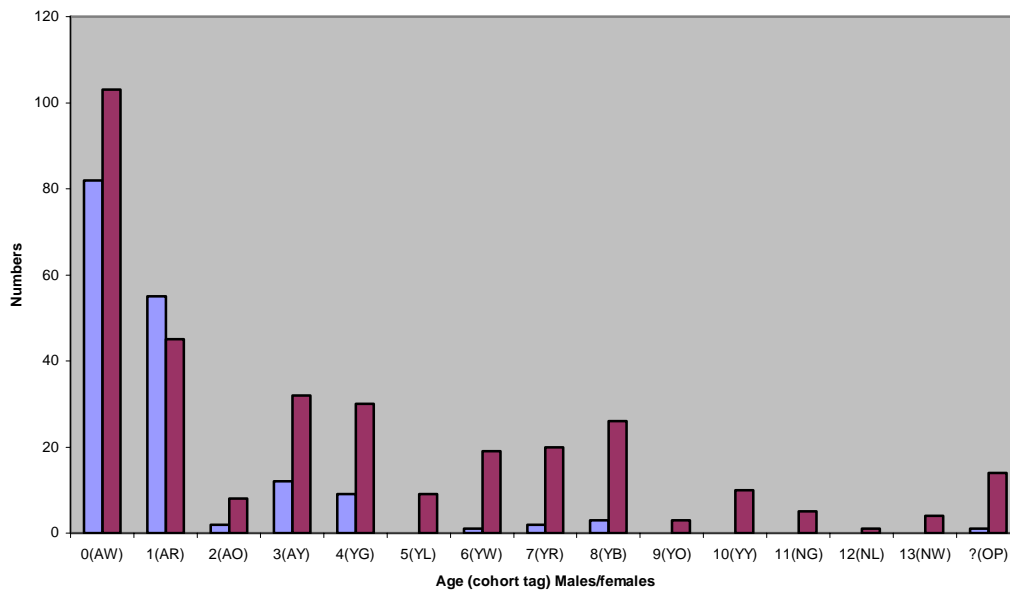


Figure 2. Age distribution of tagged Soay sheep presumed to be alive at the end of 2003.

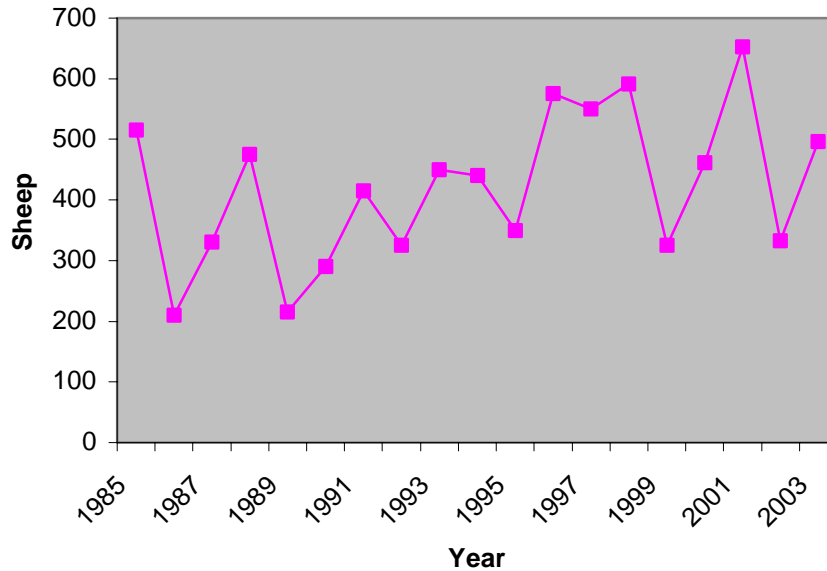


Figure 3. The number of tagged sheep regularly using the study area since 1985.

One whole-island count yielded 1568 tagged and untagged sheep on the whole island with the details displayed in Table 1. The total population had increased by 74% since summer 2002, when the count was 902. This is one of the largest increases ever recorded for a ruminant population.

Table 1. Demographic and geographic distribution of sheep observed during the count of *Hirta* on August 11th 2003. Coat colours are DW = dark wild, DS = dark self, LW = light wild, and LS = light self.

Location	Females				Males				Lambs	Total
	DW	DS	LW	LS	DW	DS	LW	LS		
Conachair/Oiseval	117	7	41	1	35	3	14	0	137	355
Mullach Bi/Cambir	169	11	74	3	49	3	14	0	215	538
Ruaival/Village	214	13	79	2	86	1	17	0	263	675
Total	500	31	194	6	170	7	45	0	615	1568

REPORTS ON COMPONENT STUDIES

New developments in estimating fitness in the wild.

Tim Coulson, Loeske Kruuk, Dave Coltman, Josephine Pemberton, & Tim Clutton-Brock.

Fitness is a critical evolutionary parameter that is used to compare individual quality, to estimate selection and to identify small-scale evolutionary change. Fitness is technically an individual's genetic representation at an arbitrary point in the future. In order to know an individual's true fitness, it would be necessary to count up all the copies of all an individual's alleles within a population at a point in time. This is currently impossible to do. Consequently, when fitness is estimated for studies of wild animals, biologists typically use measures that are believed to correlate strongly with an individual's future genetic representation. The most commonly used measures are based on the number of offspring an individual produces over its lifespan. Unfortunately, under a wide range of conditions, the lifetime production of offspring fails to capture future genetic representation, partly because some offspring may survive and transmit parents' genes into future generations, while others may die shortly after birth. Yet lifetime offspring production considers all offspring as equivalent.

We decided to see whether we could improve on estimates of individual fitness using the Soay sheep data. First, we realised that the later in life an individual's descendants are censused, the more accurate our estimate of an individual's genetic contribution to future generations. We did this by counting the number of living descendants an individual had when it died. Second, we assessed whether this number was better or worse than the average performance of animals in the population over the same time period. Finally, we took the difference between the individual's performance and the average performance and scaled this by the average relatedness of surviving descendants to the focal individual. We assumed that offspring have half of a parent's genes, a grand-offspring a quarter of a grandparent's genes and so on for future generations.

Having devised this new measure, which we call relative lifetime performance (RLP), we conducted computer simulations to see whether our new measure correlated more strongly with an individual's future genetic representation than the number of offspring produced. We found that the lifetime production of offspring correlated with fitness significantly worse than RLP; our new measure captured about 80% of actual fitness while lifetime offspring production captured only 50%. We were also able to prove mathematically that RLP is a better estimator of fitness than lifetime offspring production.

Does RLP tell us anything different about natural selection than measures based on lifetime offspring production? We found that it did: estimates of the strength of selection on longevity and age at first reproduction differed by a factor of two dependent on the measure used. Excitingly, in contrast to lifetime offspring production, RLP also suggested that fitness may be heritable – a fit parent is more likely to produce fit offspring than an unfit parent.

Investigating reproductive responses of individual Soay ewes to climatic conditions.

Dan Nussey and Loeske Kruuk.

Phenotypic traits associated with reproduction, such as birth date and birth weight, are often related to climate conditions during pregnancy. Our previous research on red deer on the Isle of Rum has shown that these relationships are often driven by the responses of individual mothers to the environment. For example, we found that a correlation between calf birth dates and the amount of rainfall in the previous autumn amongst the Rum deer is caused by mothers giving birth earlier in response to dry conditions early in their pregnancy. We were also able to show that some females responded more strongly to weather conditions than others. Variation in individual responses to the environment has rarely been documented or examined in wild populations of animals and could be related to individual quality or physiological condition, and have a strong influence on observed population dynamics.

We extended our study to look at the responses of individual Soay ewes on Hirta to the environment. Previous studies have shown that the sheep in the Village Bay population are born lighter following wet, warm and windy winter conditions (as indicated by high North Atlantic Oscillation, or NAO, index measurements). We wanted to know whether this trend was observed because individual ewes were unable to invest as much in their offspring following high NAO winters, and whether ewes responded to weather conditions in different ways. The four possible situations that could describe the way the Village Bay ewes respond to winter NAO conditions are illustrated in Fig. 4. They could either:

- (a) Show no response at all (Fig. 4a);
- (b) All respond by reducing their lambs' birth weights in response to winter NAO in exactly the same way (Fig. 4b);
- (c) Show varying responses to winter NAO conditions without reducing their lambs' birth weights following high NAO winters, on average (Fig. 4c);
- (d) Show varying responses to winter NAO conditions but reduce their lambs' birth weights following high NAO winters, on average (Fig. 4d).

We ran a linear mixed-effect model on data from the offspring of 361 ewes in the Village Bay population who had more than one lamb. The model showed clearly that following warm, wet and windy winters ewes were, on average, giving birth to lighter lambs. It also showed that there was some variation in the way that ewes respond to winter weather conditions. This suggests a pattern of response similar to that shown in Fig. 4d (circled) across these animals.

We intend to further investigate the apparent variation in the responses of the Village Bay ewes to the environment. It would be interesting to know if ewes that respond to NAO index very strongly have more surviving offspring or are more likely to survive population crashes than those that do not respond. Our work on red deer also suggests that the physiological condition of females during or before pregnancy may in some way determine their response to the environment. We would like to know if similar processes govern the responses of the Soay sheep on Hirta, by investigating whether females are less likely to respond to climate if they are reproducing at high population densities.

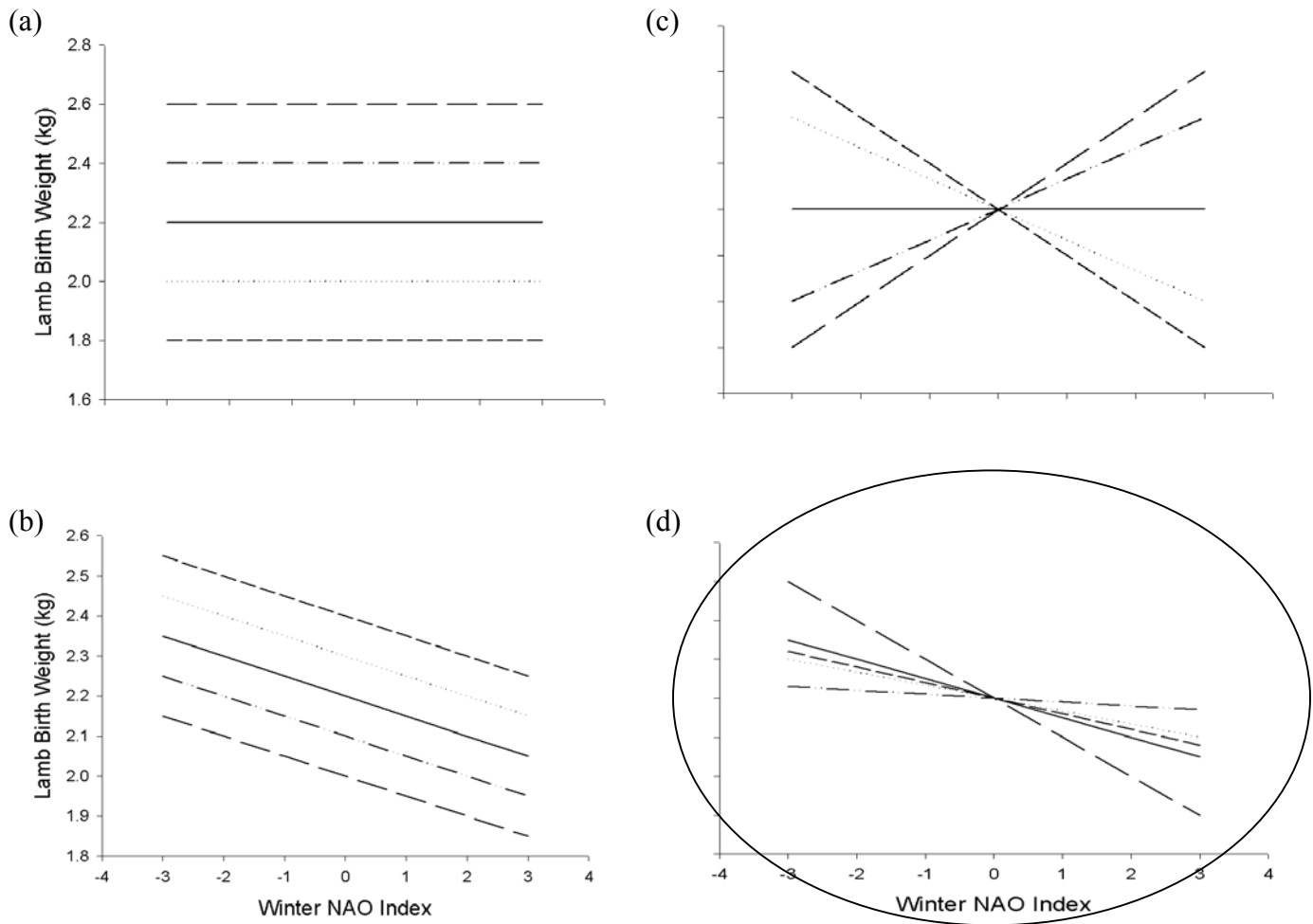


Figure 4. Line plots illustrating the four general patterns of response to winter NAO that could be observed in the Village Bay population of ewes, using data from five fictional individuals. Linear mixed-effects models revealed that a pattern similar to that shown in panel (d) (circled) is most likely to be that seen amongst these ewes for the lamb birth weight – winter NAO index relationship.

Inbreeding and neonatal survival.

Andy Overall, Kate Byrne & Josephine Pemberton.

The majority of mortalities that occur in Soay sheep are within the first year of birth (58%, Fig. 5). Death rates are particularly high during a lambs first winter (if at high density) and within the first month of birth. A previous study identified a relationship between the probability of surviving the winter and inbreeding, with less inbred individuals more likely to survive. Our study aimed to identify whether there was any

association between inbreeding and the probability of surviving the neonatal period (birth – 1st October), within which 24% of lambs die.

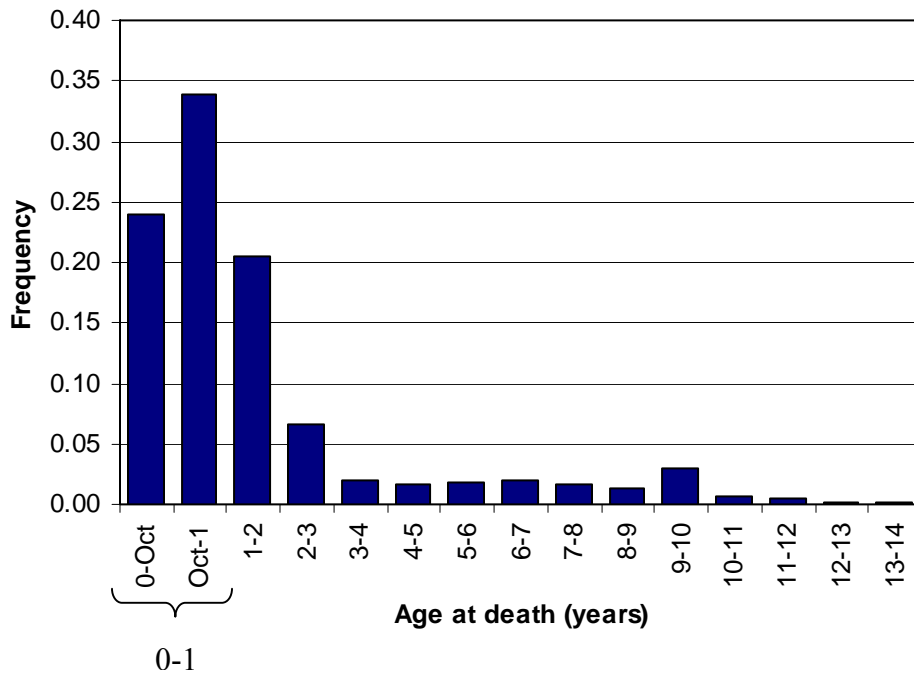


Figure 5. The age at death, in years, of Soays with the first year split into neonatal (0-Oct 1st) and overwinter (Oct 1st – 1 year) periods.

With the recent genotyping of an additional ten microsatellite markers, individuals born 1987-2002 have now been genotyped at up to 24 loci. Heterozygosity scores calculated for each individual typed at eight loci or more were used as an indirect measure of inbreeding. We found no significant differences between the mean heterozygosity of lambs that died during the neonatal period and those that survived (two-tailed *t*-test, $t_{1375} = 1.96$, $P > 0.2$). To check that an effect of heterozygosity was not obscured by other variables affecting neonatal survival, we used logistic regression to investigate the relationship between survival, population density, winter climate and individual attributes including heterozygosity (Table 2).

Table 2. Summary of minimum adequate model of neonatal survival (binomial model, response: survived, $n=1453$). Total deviance explained = 30.51%.

Term	df	% deviance explained	p	Coefficient
Birthweight	1	58.56	<0.0001	1.694
Birthyear	14	21.30	<0.0001	
Mumage	1	7.18	<0.0001	0.648
Mumage ²	1	6.00	<0.0001	-0.052
Birthday	1	4.72	<0.0001	0.056
Sex	1	2.24	0.0217	-0.190

Our analysis shows that inbreeding, as measured by heterozygosity, has no significant influence on the probability of a lamb surviving the neonatal period. Consistent with previous studies of juvenile survival of Soay sheep (Clutton-Brock *et al.* 1992), birthweight was the primary factor affecting the lamb's probability of surviving the neonatal period, with heavier, female lambs born later in the spring being the most likely to survive. Inbreeding was still of no significance when birthweight was removed from the model.

Heterozygosity as a measure of inbreeding

As no association between inbreeding and neonatal survival was observed in our study, we investigated whether heterozygosity, measured at up to 24 markers, was a suitable indicator of inbreeding. We now have 2175 individuals with known maternities and paternities assigned at $\geq 80\%$ confidence that have been typed at 8 loci or more. However, only 1.5% (33/2175) of the individuals have a non-zero inbreeding coefficient (F) derived from the pedigree. The correlation between F and heterozygosity in this case is significantly different from zero ($P < 0.05$) but very weak, $r = -0.0673$. However, this is likely to be an underestimation as the ancestry of the remaining 98.5% is not always identified to a depth sufficient to assume $F \approx 0$. By restricting our analysis to those individuals with known maternal and paternal grandparents, we lose 80% of our pedigree ($n = 426$) but the correlation coefficient is improved to $r = -0.168$ (Fig. 6).

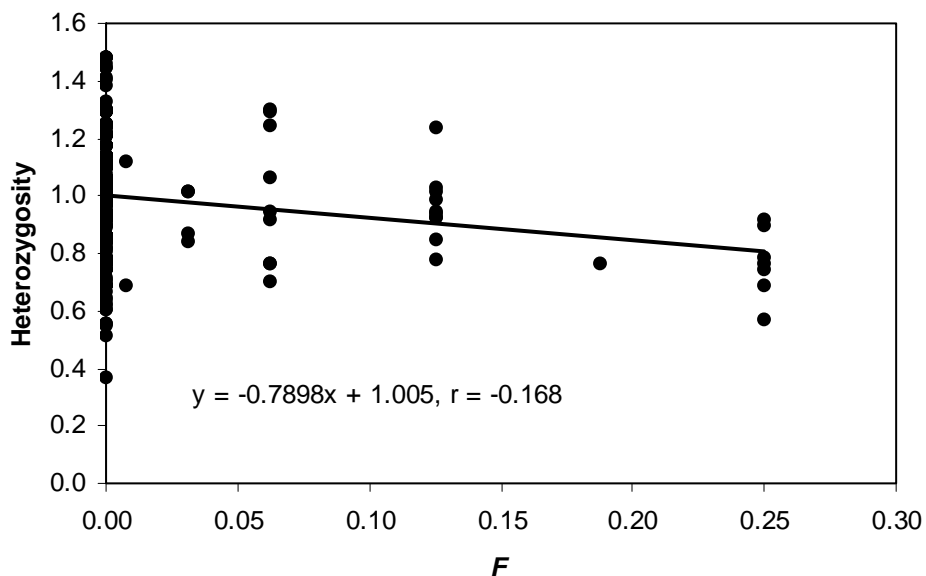


Figure 6. The relationship between heterozygosity (≥ 8 loci) and inbreeding coefficient derived from pedigrees where both maternal and paternal grandparents are known.

The relationship found between heterozygosity and inbreeding coefficient corresponds with recent theoretical prediction. This same theoretical study predicts that even with a four-fold increase in the number of loci used, the correlation will still only be about -0.42. Unfortunately, the theoretical model suggests that the correlation between heterozygosity and F will always be weak unless the population variance in F is unusually high. In the Soay sample with known grandparents this variance is in fact very low (0.001).

In conclusion, heterozygosity does not appear to explain any of the variation in neonatal survival. It would seem that, for the present, the benefits of improved genetic data are going to lie in paternity analysis, and that inference regarding the role inbreeding plays in traits, such as neonatal survival will depend upon information gleaned from pedigrees.

Quantitative genetics of birth weight and birth date in Soay sheep.

Alastair Wilson and Loeske Kruuk.

Quantitative genetics provides a theoretical framework for modelling evolutionary processes. In particular, given some knowledge of selection acting on a phenotypic trait, and some understanding of the genetic basis of trait variation, it is possible to make predictions of microevolutionary trajectories. If a trait is both under selection, and has heritable variation then it is expected to evolve. Here we constructed simple quantitative genetic models for the phenotypic traits of birth weight and birth date (which are positively correlated with each other) in Soay sheep.

Selection on each trait was characterized by using standardized selection gradients, determined as the partial regression coefficients from a multiple regression of fitness on phenotypic traits. For this purpose fitness was defined in several ways: as survival to October 1 in the year of birth, as survival to May 1 in the year after birth, as Lifetime Breeding Success (LBS, the number of offspring produced during an individual's lifetime), and as Lifetime Reproductive Success (LRS, the number of offspring produced during an individual's lifetime that survived to at least age 1).

To test for heritable variation in the traits, we estimated variance components using an Animal Model. The Animal Model is a form of mixed model in which both fixed and random effects are fitted. By using phenotypic data in conjunction with pedigree information, this allows estimation of genetic parameters such as additive genetic variance and heritability (h^2 , the proportion of total phenotypic variance that is attributable to additive genetic effects). This type of approach is particularly well suited to the analysis of free-living or natural populations, because it allows information from all different types of relationship to be used and does not require balanced data sets. For our analyses we used a pedigree structure comprised of all maternities determined observationally, and all paternities assigned from genetic data with 80% confidence or more. Heritabilities, and the genetic correlation (r_G) between birth weight and birth date, were then estimated using two models. Both models included fixed effects of sex, birth year, maternal age and whether an individual was a twin or singleton. Model 1 had the single random effect of ANIMAL resulting in partitioning of phenotypic variance into two components (additive genetic and residual variances), while in Model 2 we fitted MOTHER as an additional random effect, yielding a third, maternal component of variance. The maternal effect (m^2), is the variance due to the identity of the mother as a proportion of total phenotypic variance.

Results of the selection analysis (Table 3) indicate that there is significant directional selection acting on birth weight and birth date. For all four measures of fitness, significant positive selection gradients were found for birth weight. The positive sign

indicates that this reflects directional selection favouring heavier lambs, with increased weight associated both with increased short term viability and with longer term fitness. Later birth date was also found to provide a selective advantage by increasing early survival, though this effect was not as strong and did not translate to increased long-term fitness (as measured by LBS and LRS).

Estimated heritabilities are significantly greater than zero for birth weight and birth date, and there is a significant positive genetic correlation between the traits (Table 4). However comparison of Models 1 and 2 shows that h^2 estimates differ greatly, being substantially reduced by the inclusion of maternal effects. The presence of a maternal effect is expected to increase phenotypic similarity between siblings, and thus failure to specify maternal variance in the model will lead to inflation of h^2 (as phenotypic similarity is erroneously attributed only to shared genes).

The finding that heritable variation for birth weight is present (even after accounting for maternal effects), means that selection might be expected to cause increased birth weight over time. Furthermore, despite that the absence of significant selection on birth date (over an individual's lifetime), the genetic correlation between the traits means that increased birth date should evolve as a correlated response. However, preliminary examination of the phenotypic data suggests that there has been no significant increase in birth weight or birth date over time.

Work is currently in progress to examine possible constraints on the evolution of these traits, focusing in particular on the potential role of maternal effects. For example, there may be a balance between selective forces operating at offspring and maternal levels, with increased birth weight being beneficial to a lamb but imposing a fitness cost on the mother (through reduced future viability or fecundity). We are also examining whether the maternal effects themselves have a significant genetic component. If so, genetic covariance between direct and maternal genetic effects may have considerable implications for predictions of trait evolutionary trajectories.

Table 3. Standardized linear selection gradients for directional selection on birth weight and birth date in Soay sheep. (* denotes significant at $p < 0.05$)

Fitness	Birth weight	Birth date
Survival to October 1	+0.25*	+0.11*
Survival to May 1	+0.32*	+0.06*
LBS	+0.60*	+0.11
LRS	+0.60*	+0.08

Table 4. Estimates of heritability (h^2), genetic correlation (r_G), and maternal effect (m^2) for birth weight and birth date in Soay sheep. Standard errors are given in parentheses.

	Birth weight	Birth date
Model 1		
h^2	0.389 (0.043)	0.355 (0.036)
r_G	+0.309 (0.079)	
Model 2		
h^2	0.129 (0.047)	0.127 (0.040)
m^2	0.252 (0.023)	0.316 (0.027)
r_G	+0.586 (0.208)	

The genetic basis of quantitative trait variation: a linkage analysis approach.

Dario Beraldi, P. Visscher, Jon Slate, Allan McRae, & Josephine Pemberton.

Background and aim of the project

Most of the characteristics of an organism result from complex interactions between many genetic loci and the environment. These characteristics can be referred to as quantitative traits or QTs, and can be analysed by the methods of quantitative genetics as outlined in the previous report. In order to study the detailed genetics of these traits in terms of variation at the underlying loci, artificial experimental populations have been created and thoroughly analyzed, but little is known about the variation underlying QTs in the wild. Thanks to the inferred genealogy and collected data, the Soay sheep population has the prerequisites to shed light on the complexity of the QTs in the natural environment.

The purpose of this research is to identify the regions of the chromosomes containing genes responsible for morphological traits (birth and adult weight, hind leg length), parasite resistance, and fitness components (longevity, number of offspring).

The principle of the method is to follow the inheritance of alleles at a large number of molecular markers through a set of related individuals and then to test for a statistical association between one or more markers and the value of the trait under study. A molecular marker is a DNA sequence for which the genetic position in the chromosomes is known and which is polymorphic with a number recognizable variants or alleles among different individuals.

Progress of the work

A pedigree of more than 600 individuals including the largest paternal and maternal families (Fig. 7) has been selected from the whole Soay dataset on the basis of data availability and power of statistical analysis. The DNA of the selected individuals will be analyzed with a set of 200 microsatellite markers covering the vast majority of the sheep genome. An initial screening of eight relevant sires yielded 56 polymorphic markers

(mean of 3.5 alleles per marker, Fig. 8) distributed over seven out of the 26 chromosomes.

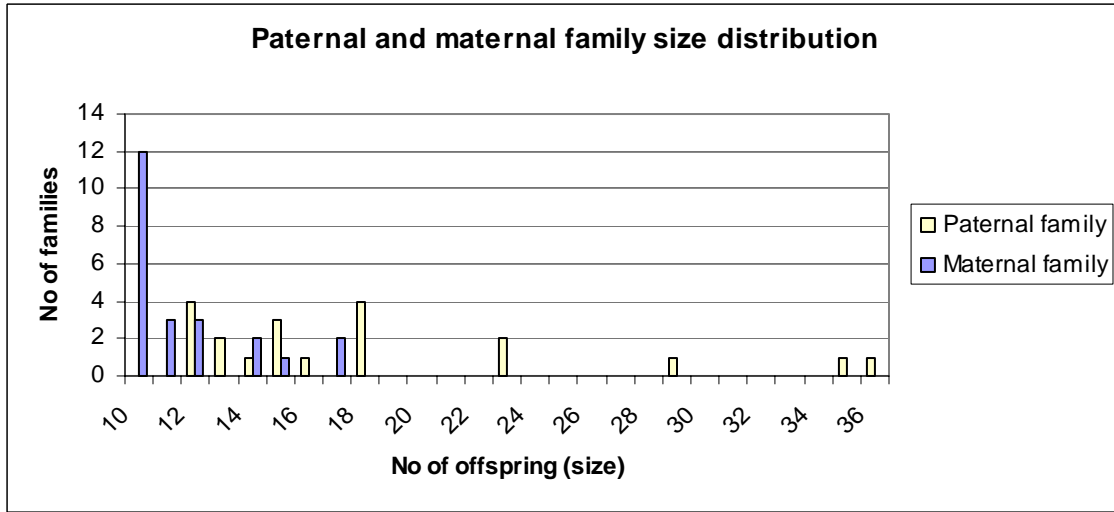


Figure 7. Count of the largest paternal and maternal half sib families on St. Kilda for which we also have data on offspring birth weight and other data.

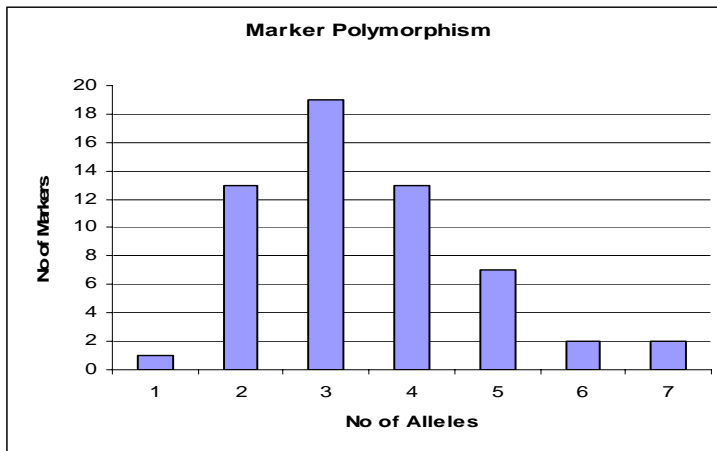


Figure 8. Polymorphism at 57 microsatellite markers. 56 markers show more than one allele.

The genetic basis of quantitative trait variation: a candidate gene approach.

Jake Gratten & Jon Slate.

As introduced by the previous report, a key goal of the Soay sheep project is to understand the genetic basis of the phenotypic variation that is observed between individual sheep and the mechanisms by which this genetic variation is maintained. A second approach to understanding this genetic variation, known as a candidate gene

study, examines genetic variation at specific genes that *a priori* might be expected to influence a trait of interest. With approximately 30,000 genes in the sheep genome, candidate gene approaches have thus far been intractable. However, in recent years researchers in New Zealand and elsewhere have discovered a handful of genes that are known to influence fecundity, coat colour, seasonality and parasite resistance in domestic sheep breeds. We have conducted a preliminary examination of these genes in Soay sheep to determine whether any of them also play an important role in Soay phenotypic variation. This project has two central aims: (1) to rapidly identify the genes that are responsible for fitness variation in a “natural laboratory” and (2) to conduct the first systematic investigation of the feasibility of a candidate gene approach in a wild organism.

Fecundity

Fecundity is an important component of fitness in wild populations. We examined two genes that are known to control ovulation rate in domestic sheep, Booroola and Inverdale/Hanna. High prolificacy in Booroola sheep is due to a mutation in a dominant gene on sheep chromosome 6. Each copy of the Booroola mutation increases ovulation rate by about 90%. High prolificacy in Inverdale and Hanna sheep is due to two different mutations in a dominant X-linked locus. Ovulation rate of ewes that are heterozygous for either the Inverdale or Hanna mutation is ~1.0 higher than in wildtype ewes, whereas ewes that are homozygous for either mutation are infertile. We sequenced Booroola and Inverdale/Hanna in Soay ewes with high (n=3) and low (n=3) fecundity. We found no sequence variation between high and low fecundity Soays in either gene. The mutations known to cause high prolificacy in Booroola and Inverdale/Hanna sheep are not segregating in Soays.

Coat colour

Colour morph in Soays is thought to be controlled by two diallelic autosomal loci, Brown at which dark (B^+) is dominant to light (B^b) and Agouti at which wild (A^+) is dominant to self (A^a). Coltman & Pemberton (2002 Kilda Report) showed that this two locus model is a good fit to the observed inheritance of dark/light and wild/self in Soays. A likely candidate for the Brown locus controlling dark/light colouration is melanocortin receptor 1R (MC1R), because mutations are known to affect colouration in humans, mice, pigs, horses, cattle and domestic sheep. We sequenced the relevant region in several dark wild, light wild, dark self and light self Soays. We identified three variable positions in this region but all were synonymous (i.e. non-coding). Coding polymorphisms in MC1R that are known to be responsible for dark/light colouration in other mammals are not segregating in Soays.

Timing of oestrus

The timing of reproduction in Soays, like the majority of temperate mammals, is seasonal, characterised by a defined period of oestrus between late October and early December. Soay ewes do not ovulate outside of this period, which is known as the rut, but there is individual variation in the timing of oestrus; some females cycle consistently earlier than the population mean, and others consistently later. The timing of oestrus may have important fitness consequences in Soays because it may be correlated with birth date, birth weight and fecundity. We tested for an association between the timing of oestrus in Soays and DNA sequence variation in melatonin receptor 1a (Mel_{1a}). This receptor is a strong candidate for reproductive scheduling because a polymorphism

within it is known to be associated with seasonality in Merinos d'Arles sheep, a breed in which some ewes are seasonal and others spontaneously ovulate during the anoestrus period. Soay ewes that cycled consistently earlier (n=6) or later (n=4) than the population mean in at least four years were identified and sequenced. This work revealed that the polymorphism in the relevant region is also segregating in Soays. The frequencies of genotypes in early and late breeding Soay ewes was remarkably similar to that reported for seasonal and non-seasonal Merinos d'Arles ewes (Table 5); in both cases the A/A genotype was associated with early rather than late breeding. At this stage we have too few data to determine if Mel_{1a} genotype is significantly associated with the timing of oestrus in Soays. Next we will screen a larger sample of individuals using a rapid genotyping method.

Table 5. Genotype frequencies at position 706 of Mel_{1a} exon II in Soays and Merino d'Arles sheep*.

Breed and reproductive timing	Genotype at position 706 of Mel _{1a} exon II		
	G/G	G/A	A/A
Soays early (n=6)	0.33	0.50	0.17
Soays late (n=4)	0.50	0.50	0.00
Merinos D'Arles seasonal (n=35)*	0.29	0.43	0.29
Merinos D'Arles non seasonal (n=36)*	0.53	0.47	0.00

*Data from Pelletier *et al.* (2000).

Parasite resistance

The main parasites of both Soays and domestic sheep are gastrointestinal (GI) nematodes. Infection by these parasites is known to be associated with over-winter survival in Soays and is likely to have an important impact on fitness. Resistance to GI nematode infection is a complex trait influenced by many loci. In Soays associations have been found between faecal egg count (FEC) and adenosine diaminase (Ada), the major histocompatibility complex (MHC) and gamma interferon (IFN- γ). The best studied of these candidate resistance genes in sheep is IFN- γ , a locus that plays a role in switching immune function between defence against intracellular pathogens and extracellular parasites. This locus was first identified by whole genome scans in Australian and New Zealand domestic sheep, which independently identified a major resistance gene on chromosome 3 at or near the location of IFN- γ . Subsequent studies in NZ flocks revealed a significant association between IFN- γ variation and nematode resistance, although there were no amino acid differences between resistant and susceptible alleles. This suggests that the resistance effect in NZ flocks is due to either: (1) a regulatory polymorphism that results in differential expression of resistant & susceptible haplotypes, or (2) an as yet uncharacterised locus closely linked to IFN- γ . In order to determine if the effect observed in Soays is due to functional differences between resistant & susceptible alleles, we have sequenced the complete IFN- γ gene in several Soays matched for age, sex, heft and sample date, but differing in nematode infection level. This work confirmed the absence of functional mutations between Soay sheep with high and low FEC. All variable sites identified in NZ flocks are also segregating in Soays, in addition to four novel polymorphisms not observed in the NZ domestic sheep. This result suggests that variation in IFN- γ has been maintained for long periods in the face of genetic drift and both artificial and natural selection. In collaboration with AgResearch, NZ we will

characterise the extent of linkage disequilibrium and haplotype sharing in and around IFN- γ in Soays and NZ domestic flocks to clarify whether this locus is responsible for the resistance effect.

Costs of reproduction and parasitism in Soay ewes.

Louisa-Jayne Tempest & Ken Wilson.

Over the lifetime of an individual, decisions are constantly being made about if, when, and how much to breed. These decisions arise as a result of trade-offs between life-history traits, for example lifespan and fecundity. Trade-offs may occur when two traits are limited by the same resource, i.e. one trait can only be increased at the expense of another. The cost of reproduction is a trade-off between current and future reproduction, and we are currently trying to understand how parasites interact with the costs of reproduction in Soay sheep on St Kilda.

Parasitism varies seasonally and is measured by faecal egg count (FEC) of strongyle nematodes (i.e. the density of parasite eggs in the faeces). Domestic sheep show a rise in FEC around the time of lambing. This is termed the Peri-Parturient Rise (PPR). It is believed that hormones released during lactation (e.g. prolactin) cause immunosuppression, which:

- Triggers maturation of arrested larvae
- Allows infection by new larvae on the pasture
- Prevents expulsion of egg-producing adult worms

These factors result in a rapid increase in FEC around the time of lambing in domestic sheep. However, the PPR is absent in domestic ewes that are prevented from breeding, and is much lower in those that lose their lamb at birth. In comparison, Soay sheep also show a rise in FEC at lambing; however ewes that successfully wean a lamb have a lower PPR than those that do not. Male Soay sheep can also suffer a spring increase in FEC during years of high population density, suggesting that stresses associated with food shortage may also be involved with this phenomenon. Although there is evidence of an association between the spring rise in FEC and reproduction, it seems likely that there are confounding variables that mask it in Soay sheep.

In an attempt to clarify the relationship between spring parasitism and reproduction in Soays, we used spring FEC data collected for known ewes over 15 years. Analysis of FEC (log₁₀-transformed) was done using GLMM mixed effects models in the statistical package S-Plus. Analyses are not yet complete, however things are becoming clearer. As Soay ewes become older their parasite burdens (and the peak of their PPR) declines ($F_{1,550} = 148.0, p < 0.0001$). Spring FEC also decreases with body weight ($F_{1,96} = 7.74, p = 0.0065$), although weight-related differences in FEC are much more apparent in younger ewes ($F_{1,96} = 12.0, p = 0.0008$). *Observed* FEC data still suggest ewes that raise a lamb have a lower PPR than ewes that don't. However, after *accounting for age and weight*, it appears that ewes that raise a lamb successfully do have a relatively higher PPR than those ewes that do not ($F_{1,550} = 17.0, p < 0.0001$, Fig. 9)

Thus, Soay ewes appear to suffer a cost of reproduction in terms of increased susceptibility to nematode parasitism, similar to domestic sheep. However there are more variables confounding the effect. Soay sheep differ markedly in their body condition entering the breeding season and this affects their reproductive decisions. Ewes in poor condition may lose their lamb either during gestation or just after parturition. This enables them to avoid the parasitism “cost of reproduction” that lactating ewes bear. This work is ongoing and requires further consideration of the factors that may contribute to ewe body condition and their reproductive decisions.

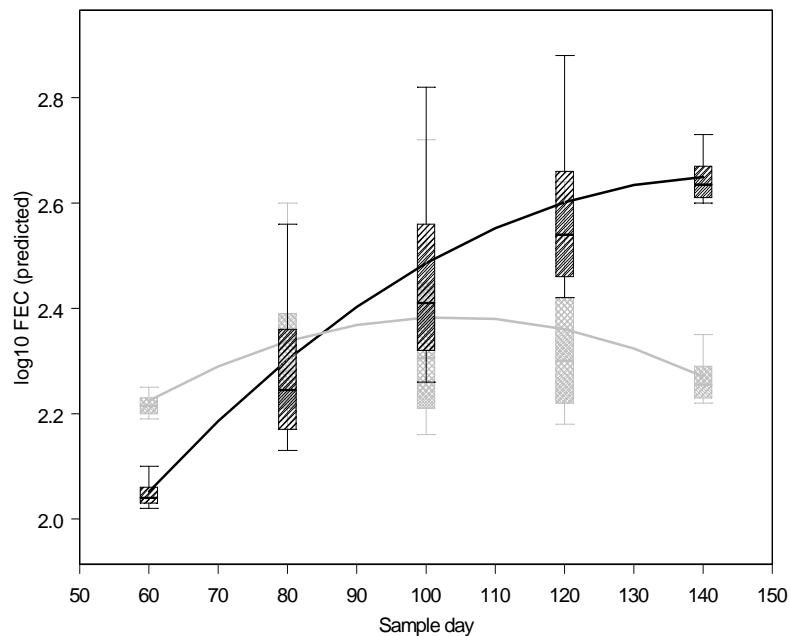


Figure 9. Predicted FEC (controlling for age and weight) for ewes that successfully weaned a lamb (black line), and for ewes that failed to raise a lamb (grey line). Box plots show the range of predicted FEC. Sample day 1 = January 1st.

Epidemiology of the parasitic protozoa of St. Kilda Soay sheep.

Barbara Craig, Jill Pilkington and Josephine Pemberton.

Since thirteen species of parasitic protozoa were identified from St. Kilda Soay faecal samples, in August 2001, their prevalence and intensity has been monitored in the subsequent summers. So far, simple χ^2 analysis has been used to test for between group differences in prevalence. The results give an epidemiological picture using prevalence of each species, in lambs and adults and in males and females, at a point of high (2001), low (2002) and intermediate (2003) host population density.

Regardless of population density, lambs always had significantly higher prevalence of *Giardia duodenalis* and all the *Eimeria* spp. than adults, except *E. granulosa* for which adults always had higher prevalence. Interestingly, prevalence of *Cryptosporidium parvum* stayed the same in adults each year but appeared to vary positively with population density in lambs. This meant that the higher prevalence in lambs than adults in 2001 was reversed in 2002, with no significant difference in 2003 (Fig. 10).

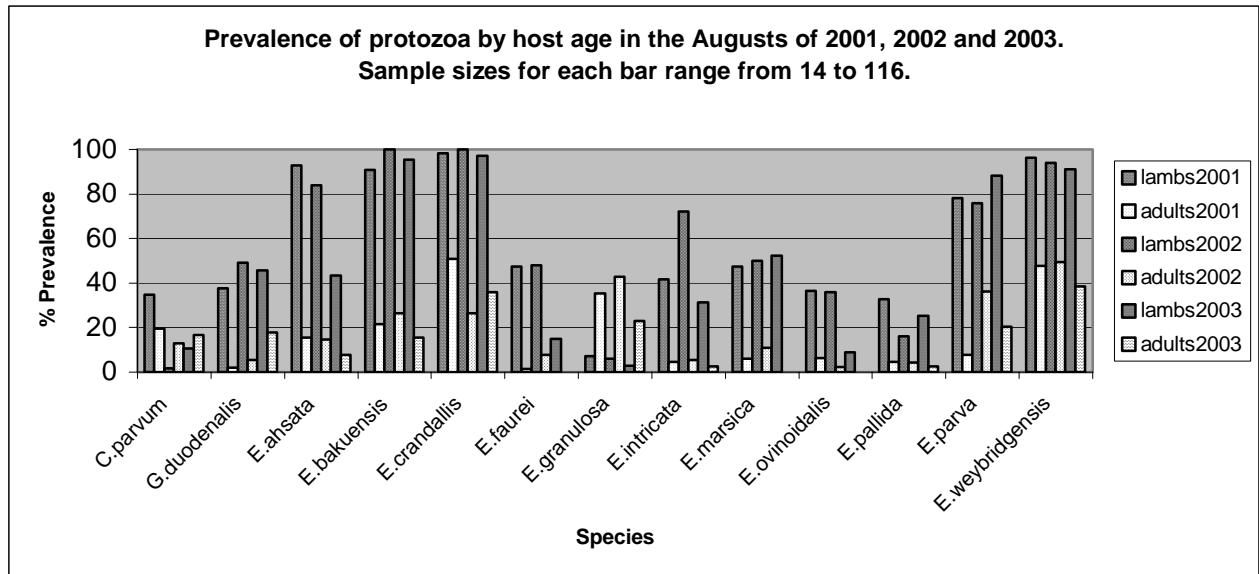


Figure 10. Generally, prevalence of each protozoan species was significantly higher in lambs than adults in all years ($p < 0.05$), except for *E. granulosa* which was higher in adults ($p = 0.000$). *C. parvum* was more prevalent in adults than lambs in 2002 ($p = 0.01$) but did not differ between age classes in 2003.

In each August, males had a higher prevalence of each species, except *E. granulosa*, which was significantly higher in females in 2002. Following the population crash that year, the male bias became even more evident for most of the other species. (Fig. 11)

Six of the *Eimeria* spp. did not significantly change their respective prevalence in lambs and adults across the three Augusts. These consistent species were *E. bakuensis*, *E. crandallis*, *E. marsica*, *E. pallida*, *E. weybridgensis* and *E. granulosa*. There were five species which showed no change in prevalence in adults but changed in lambs, of these *E. ahsata*, *E. faurei* and *E. ovinoidalis* decreased significantly in 2003 ($P = 0.000$). The decrease, then increase, of *C. parvum* in 2002 and 2003 respectively, was an exactly opposite pattern to *E. intricata*. Two species; *E. parva* and *G. duodenalis*, did not change in lambs but increased significantly in adults in 2002 ($p = 0.000$) and 2003 ($p = 0.025$) respectively.

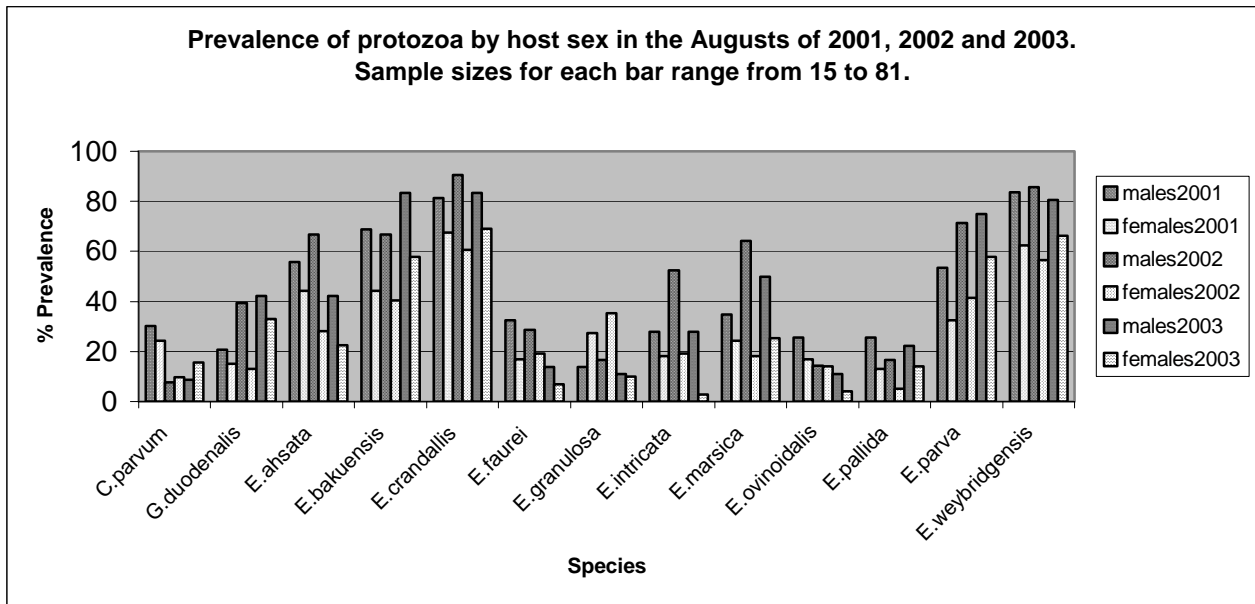


Figure 11. Generally, prevalence of each protozoan species was higher in males, the difference becoming greater following the population crash in 2002. Only *E. granulosa* was more prevalent in females, in 2002 ($P=0.026$).

These initial results indicate that there is generally a juvenile and male bias in infection with most of the protozoan species, with the exception of *E. granulosa*. Population density is an important factor in the transmission of *C. parvum* in lambs and the increase in prevalence of this species, with the concomitant decrease of another, raises interesting questions as to the competitive interactions between the protozoa in the sheep intestines.

Understanding helminth parasite diversity.

Barbara Wimmer, Barbara Craig, Josephine Pemberton & Jill Pilkington.

The objective of this study is to identify the mechanisms by which a host might achieve resistance to parasites. Two working hypotheses for the possible mechanism of achieving parasite resistance have been proposed:

- 1) Resistance is achieved by keeping the overall number of nematodes low irrespective of species.
- 2) Resistance is achieved by reducing the number of particular species.

Parasitic nematodes are very difficult to distinguish and it is almost impossible to identify eggs down to species level. As a consequence, faecal egg counts are commonly pooled counts for most or all species, so at present we cannot distinguish whether, for example, a low FEC represents a low infection of all species or a low infection of a usually common species. A fast and reliable species identification method is the first step needed to approach this question. A DNA-amplification based assay, using species-specific sequences in the ribosomal DNA was developed, starting with morphologically identified adult worms. With the developed markers, nine species from six different families of parasitic nematodes found in the Soay sheep can be identified: *B. trigonocephalum* (Bt)

(*Ancylostomidae*), *C. ovina* (Co) (*Chabertiidae*), *D. filaria* (Df) (*Dictyolcaulidae*), *N. battus* (Nb) and *N. filicollis* (Nf) (*Molineidae*), *T. circumcincta* (Tc) (this includes *T. davtiani* and *T. trifurcata*), *T. axei*, *T. vitrinus* (*Trichostrongylidae*), *T. ovis* (*Trichuridae*).

One primer of each primer pair was fluorescently labelled to enable the use of an ABI 3730 DNA analyser and thus increase the through-put of samples. The use of different fragment lengths combined with different fluorescent labels allowed the detection of all nine species within a single capillary simultaneously.

The test procedure was then applied to faecal egg harvests from 124 individual hosts (31 female lambs, 40 adult females, 30 male lambs, 23 adult males), which were sampled in April 2003. The results are shown in Fig. 12. Lambs harboured on average more parasite species than adults (female lamb = 5.7, female adult = 3.65, male lamb = 5.9, male adult = 3.2) and lambs also showed a higher prevalence for most species. This was not unexpected as lambs are supposed to be less immuno-competent than adults. But the analysis also showed that the lower prevalence in adults is probably achieved by selective suppression of certain species. In particular, *B. trigonocephalum* and *N. battus* are present in almost every single host individual irrespective of sex and age, whereas especially *D. filaria*, *C. ovina*, *T. axei* and *T. vitrinus* are significantly less common in adults than in lambs. The difference between the sexes within an age class was not significant although adult females showed a tendency for higher prevalence than males especially for *T. circumcincta* and *C. ovina*. This very likely reflects the periparturient rise at the time of year these sheep were sampled (see previous report).

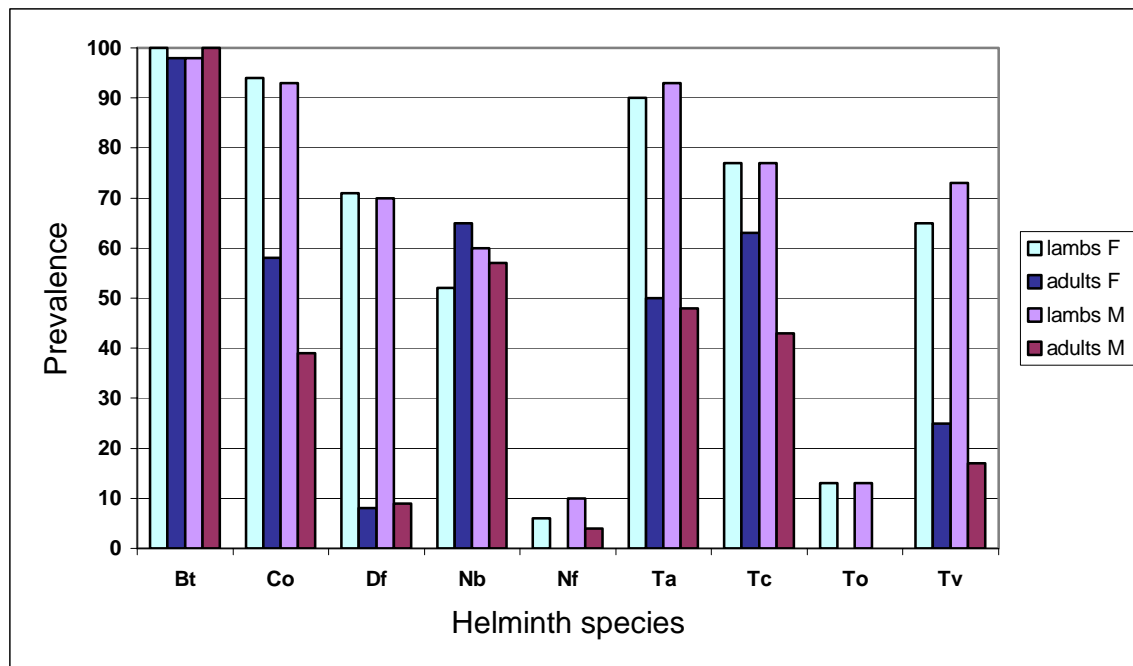


Figure 12. Prevalence of eggs of nine helminth species in 3g faecal samples from four sex-age classes of Soay sheep in April 2003 as determined by species-specific ITS-2 markers. Codes: *Bunostomum trigonocephalum* (Bt), *Chabertia ovina* (Co), *Dictyocaulus filaria* (Df), *Nematodirus battus* (Nb), *Nematodirus filicollis* (Nf), *Trichostrongylus axei* (Ta), *Teladorsagia circumcincta* (Tc), *Trichuris ovis* (To), *Trichostrongylus vitrinus* (Tv). N = 31, 40, 30, 23 for each host sex-age class respectively.

Future developments of this work will include using a technique called real-time, or quantitative, PCR to quantify the number of eggs of each species, and improvements to the protocol allowing the method to work on frozen faecal samples rather than egg harvests, which are time consuming to do.

Vegetation.

Mick Crawley.

The vegetation survey in March 2003 was unusual in almost every way. The vegetation had never been so tussocky (mean 23% tussock cover; see Fig. 13)



Figure 13. *The sward below Cottage 1 in March 2003.*

Mean sward height of the inbye grassland was the greatest we had ever recorded (5.68 cm compared with a long-term March average of 3.77cm), and total available grass biomass in the inbye was at peak levels (3.70 g 0.04 m⁻² against the long-term average = 2.66 g). This meant that the prediction of August population based on total edible biomass (4.10 g) was off the scale.

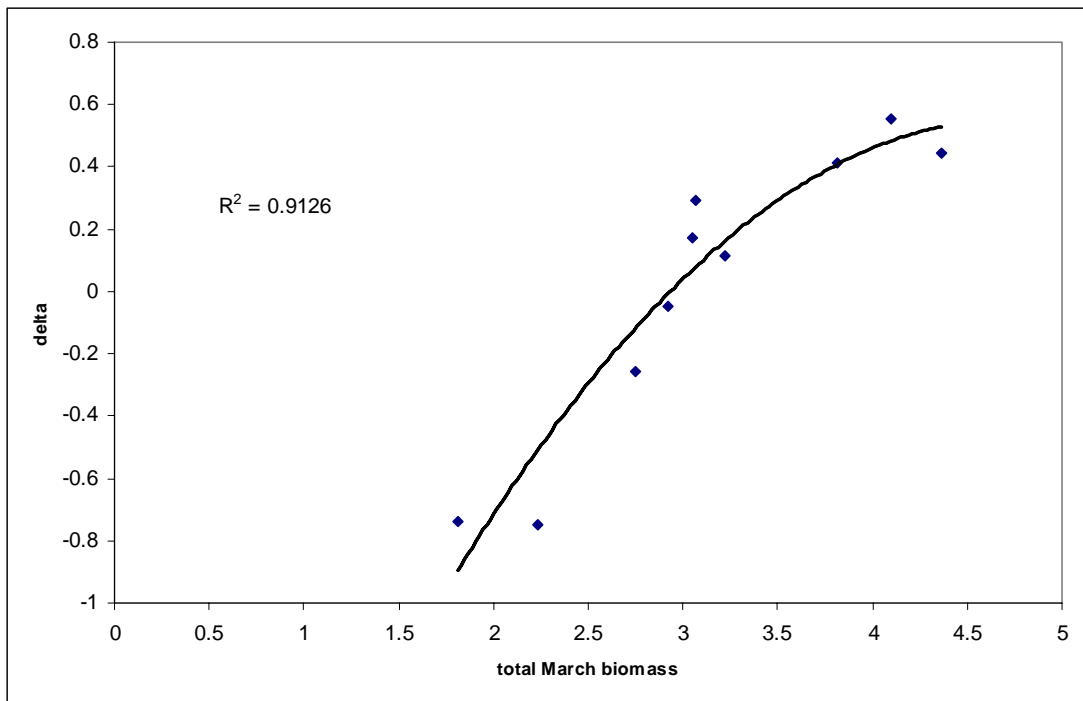


Figure 14. The relationship between year-to-year population change (δ , measured as $\ln(N_{t+1}/N_t)$) and the total biomass of edible material (g dry weight per 0.04 m^2 , excluding bryophytes and dead organic matter) in the inbye grasslands. The March 2003 data point is second from the right, predicting the highest ever population increase (solid line) but even this was surpassed by the actual outcome (data point at $\delta = 0.553$).

This led to a prediction of $\delta > 0.44$ for the period 2002-3. The sheep population in August 2002 had been 902, so we predicted a count in August 2003 of more than 1400 animals ($902 \cdot \exp(0.44)$). As it turned out, the population had increased even more than this, and we counted 1568 sheep in August 2003 (see Fig. 14), which gave $\delta = 0.553 = \ln(1568/902)$, one of the biggest r values ever recorded for an ungulate population anywhere in the world. Despite this dramatic increase in sheep numbers, there was plenty of grass in August, so no crash is predicted 2003-4 on the basis of food availability (on the contrary, August 2003 grass per sheep predicts $\delta = +0.20$, which would mean 1913 sheep in August 2004). An outstanding year, indeed.

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...and the book? It will be in the CUP warehouse in February (allegedly).

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APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK

Personnel Changes

Dan Nussey, PhD student with Loeske Kruuk in Edinburgh, who has mainly analysed plasticity in red deer data to date, is now also analysing Soay sheep data. Dario Beraldi, Jake Gratton, Alan McRae, Jon Slate and Peter Visscher have joined or rejoined the project in a collaborative study to locate quantitative trait loci between Edinburgh and Sheffield.

Schedule of work on St Kilda

Winter - Spring

From March 14th until May 13th, Jill Pilkington, and four volunteers carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 217 lambs were born to 192 ewes; these figures include 25 sets of twins (23 ewes held both lambs, 2 lost one twin). 148 lambs (68 male and 80 female) were caught and tagged; a further 22 lambs died before any tagging attempt.

Summer

Jill Pilkington and two volunteers returned to Hirta on July 15th to carry out ten population censuses, conduct mortality searches (yielding 5 tagged dead animals), and prepare for the main catch-up of study area sheep. The catch-up took place from August 9th – 21st, was led by Josephine Pemberton, and conducted by a team of 11 other project members and volunteers. 289 sheep were caught and processed, of which 117 were lambs (51 males and 66 females), 62 were yearlings (33 male and 29 females), 12 were adult males, and 98 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 58 Sheep were retagged because of damaged or missing tags. 44 previously untagged sheep (41 lambs, 1 yearlings and 2 adults) were caught and processed. Jill Pilkington, Barbara Craig, Louisa Tempest and one volunteer remained on Hirta until 2nd September to complete parasite counts and vegetation monitoring. During the catch Dr. Neil Watt, Home Office inspector for Edinburgh University under the Animals (Scientific Procedures) legislation, visited to observe our activities. Licence holders can expect to be inspected at any time, but this was the first time for us.

Autumn

From October 17th to December 5th Jill Pilkington, Brian Preston and seven volunteers monitored the mating period, carrying out focal watches, capturing and processing 44 incoming tups and carrying out seven population censuses. Four dead sheep was found. Faecal samples were also collected for parasitic egg counts and hormonal analysis.

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