ST. KILDA SOAY SHEEP PROJECT: ANNUAL REPORT 2007

J.G. Pilkington[1], S.D. Albon[2], D. Beraldi[1], D. Childs[6], T.H. Clutton-Brock[3], T. Coulson[4], B. Craig[1], M.J. Crawley[4], T. Ezard[4], J. Gratten[6], A. Hayward[1], S. Johnston[6], O. Jones[4], L. Kruuk[1], A.F. McRae[9], B. Morgan[7], F. Pelletier[4], J.M. Pemberton[1], M. Rees[6], M.R. Robinson[1], J. Slate[6], I.R. Stevenson[8] G. Tavecchia[7], L-J. Tempest[1], P. M. Visscher[9], A. Wilson[1], K. Wilson[5].

[1]Institute of Evolutionary Biology, University of Edinburgh.
[2]Macaulay Institute, Aberdeen.
[3]Department of Zoology, University of Cambridge.
[4]Department of Biological Sciences, Imperial College.
[5]Department of Biological Sciences, Lancaster University.
[6]Department of Animal and Plant Sciences, University of Sheffield.
[7]Institute of Maths and Statistics, University of Kent at Canterbury.
[8]Sunadal Data Solutions, Edinburgh.
[9]Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.

POPULATION OVERVIEW

The sheep population on Hirta entered 2007 at a moderately high level and, as a result, there was a slightly higher level of mortality than normal in a non-crash year. 140 tagged sheep were found dead within the study area between March and May of 2007. Lambing began on the 22nd of March with 71.1% of lambs born surviving (Fig. 1).

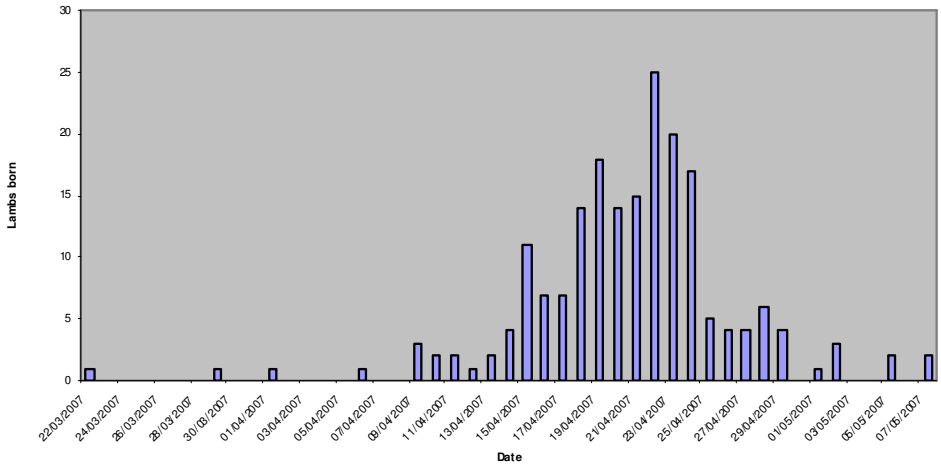

Figure 1. *The temporal distribution of lamb births during 2007.*

In December 2007, 653 tagged sheep were believed to be alive on Hirta, of which 447 regularly used the study area, a total decrease of 0.4% using the study area since 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.

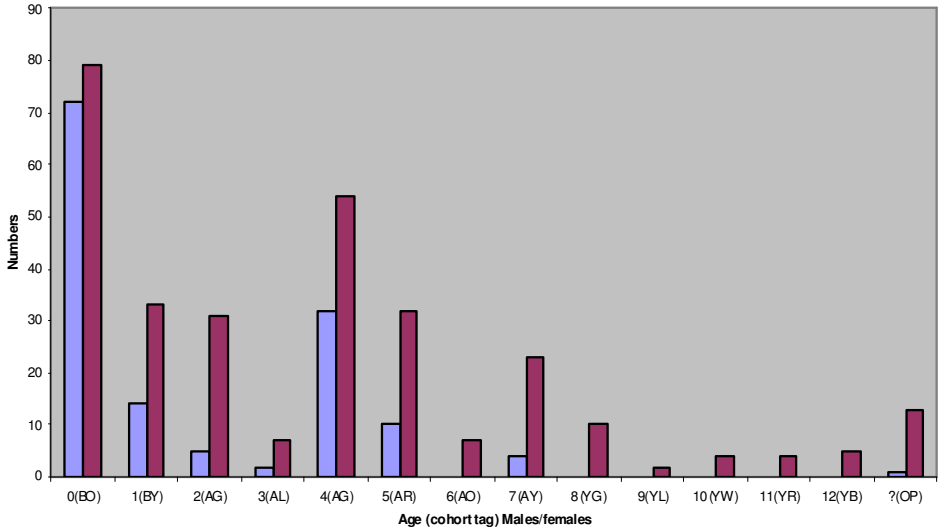

Figure 2. *Age distribution of tagged Soay sheep presumed to be alive at the end of 2007.*

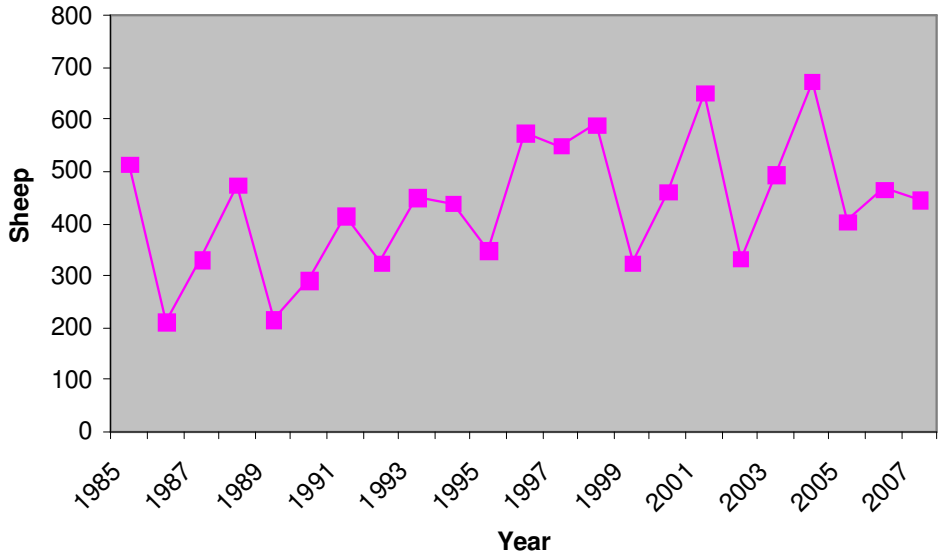

Figure 3. *The number of tagged sheep regularly using the study area since 1985.*

One whole-island count yielded 1538 tagged and untagged sheep, with the details displayed in Table 1. The total population had decreased by just over 14% since summer 2006, when it was at 1794. This gives a delta (calculated as ln (N_{t+1}/N_t)) of -0.155.

Table 1. *Demographic and geographic distribution of sheep observed during the count of Hirta on August 17th 2007. 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	154	7	52	1	57	2	19	0	122	**414**
Mullach Bi/Cambir	240	16	71	5	44	4	11	0	236	**627**
Ruaival/Village	178	9	70	2	32	0	15	0	191	**497**
Total	**572**	**32**	**193**	**8**	**133**	**6**	**45**	**0**	**549**	**1538**

REPORTS ON COMPONENT STUDIES

Vegetation.

Mick Crawley.

One of the central issues in understanding plant-herbivore dynamics is attributing cause and effect. For instance, there are clear direct effects of sheep grazing on vegetation (more sheep less grass), but there are also more subtle effects of plant dynamics on the sheep population that do not necessarily show up in any analysis of sward height or biomass. For example, increased plant productivity can allow increased offtake by sheep (and hence improved survival or lambing success) without any change in sward height.

A recurring question (on wet days in Cottage One, at least) is whether we can predict sheep population change (delta = ln(N(t+1)/N(t), where N = number of sheep on Hirta and t, t+1 are two successive years) from measurements made on vegetation alone? If so, then what measure of vegetation should we use (e.g. sward height, biomass of available green food, or something else)? And what sampling time gives the best prediction of sheep dynamics (e.g. August at the beginning of the period, March in mid-period when mortality is occurring but before lambing, or August at the end of the period)? The following graphs compare these six cases. The top row shows sheep population change (delta, between years *t* and *t*+1), as a function of mean sward height (cm) in August of year *t,* in March of year *t*+1 and in August of year *t*+1. The bottom row shows the relationship for the same three prediction times, but with green food biomass (g per 20x20cm) as the explanatory variable rather than sward height.

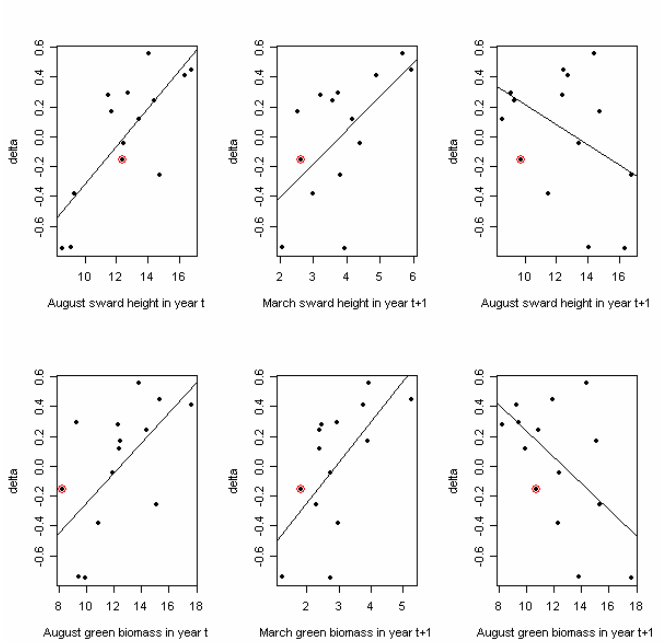

Figure 4. *Change in sheep population (delta = ln (Nt+1/Nt)) as a function of sward height (top row) or green biomass (bottom row) for data collected at three times: August(t) left, March(t+1) middle or August(t+1) right. The regression lines use data from the period 1993-2006. The circled dots show the data for change in 2006-2007. The closer the circled dot to the regression line the better the predictive value of the model. Sward height is in cm and green biomass in g per 20x20cm quadrat.*

The regression lines were calculated by leaving out the data from 2006-2007, and the circled data point shows the actual population change between August 2006 and August 2007, when the whole-island population declined from 1795 to 1538 (delta = -0.155). If the circled point fell on the line, the model would have predicted delta exactly. The vertical distance between the point and the line is a measure of the imperfection of the prediction. Of course, there is a lot of scatter in all the relationships, and the best linear fit has an r-squared of just 0.40 (delta as a function of March green biomass). As often happens, the plants were better predictors of sheep numbers than were the scientists in 2007. For instance, biomass of *Holcus lanatus* predicted the whole island count to within one individual (1537 vs. a count of 1538). But this was obviously a fluke, because in most years, *Holcus* is a lousy predictor of delta.

Of the various measurements of vegetation, the green biomass in March is the best predictor of sheep population dynamics. It is both a reflection of sheep numbers (integrated grazing pressure since grass growth stopped in the previous autumn), but it is also a cause of population change, determining the rate of starvation when there is too little food to go round. Both March sward height and March green biomass predicted substantially bigger declines in sheep population (delta.hat = -0.265 and -0.302 respectively) than was observed (delta = -0.155). We would also have expected bigger-than-average declines because there was very little over-winter grass growth in 2006-07: the pyramids contained mainly dead organic matter in March 2007 and there was little green leaf material. So this was a year when the sheep did much better than all of the plant indicators suggested, and the weather in winter 2006-07 was clearly benign. With Ana Bento (our new NERC PhD CASE student with IC and Macaulay), we are about to begin a detailed study of the weather variables that are most important in explaining the fluctuations in sheep population and primary productivity.

How do different environmental conditions influence population dynamics?

Thomas Ezard.

There are many ways in which the dynamics of populations can be summarised. One statistic that has recently been the focus of theoretical research is known as the long-run stochastic growth rate. This captures a type of average of the proportional change in population size from one year to the next given a range of specific assumptions. Biologists like this statistic as it is a powerful and insightful way of characterising how a population increases or decreases in the wild. Its principal advantage is that enables the environment to change from one year to the next.

The way that the environment fluctuates over time can influence the value of the long-run stochastic growth rate. For example, if the weather one year is a good predictor of weather in the following year, then the long run stochastic growth rate is likely to have a different value than if the weather one year is independent of the weather in previous years. Furthermore, the sequence of different types of years alters the way that survival or birth rates of different age classes contribute to the long-run stochastic growth rate.

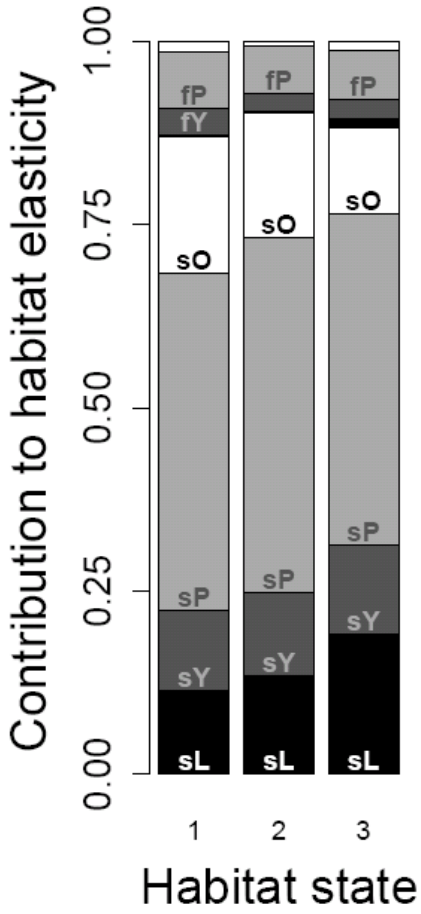

Figure 5. *Different parts of the population make different contributions to population growth dependent upon how good/bad the environment is. The eight demographic rates are composites of: s survival; f fecundity; L lambs; Y yearlings; P prime-aged individuals; O oldest individuals. The demographic rate is indicated by letters only if the contribution is greater than 3%. L are shaded black; Y: dark grey: P: light grey and O: white, such that the stacked elasticities are (from bottom to top) in the order sL, sY, sP, sO, fL, fY, fP and fO.*

I calculated the long-run stochastic growth rate for the Soay sheep and was able to show that different sequences of winter weather and food availability (measured as the height of the grazing sward) influenced the association between age-specific survival and birth rates and the long-run population growth rate. In short, in good environments lamb survival contributed much more to the long-run stochastic growth rate than in years when population density was high and the sward was low (little food). As young individuals contributed more to the long-run stochastic growth rate, older individuals contributed less (Fig. 5). These results suggest climate change could well influence the population dynamics of the sheep, and, possibly, even the way that natural selection operates.

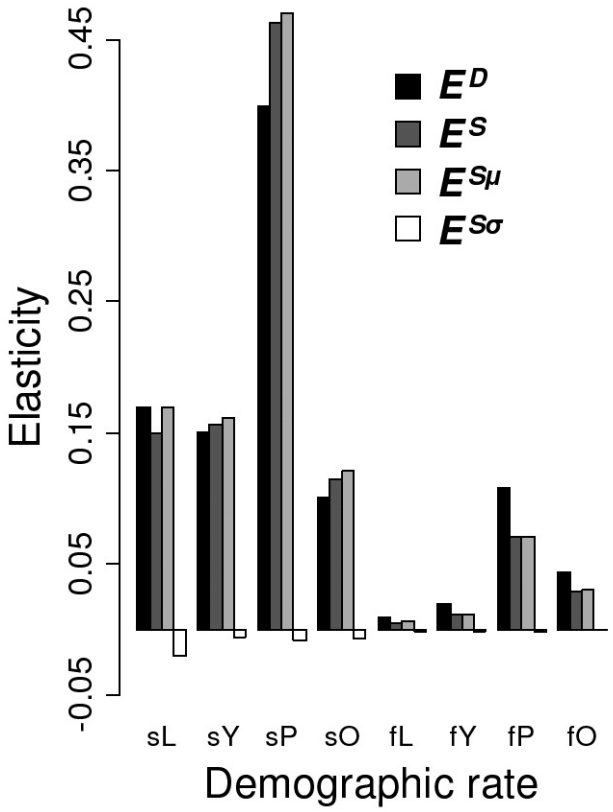

Figure 6. *Simpler methods that neglect environmental change (E^D) approximate results obtained using the more complicated long-run stochastic growth rate (E^S, $E^{S\mu}$ & $E^{S\sigma}$). The eight demographic rates are composites of: s survival; f fecundity; L lambs; Y yearlings; P prime-aged individuals; O oldest individuals.*

The method used is mathematically and computationally challenging. There are a several other methods that are much easier to use, however, they only approximately describe the long-run stochastic growth rate (Fig. 6). Simpler methods can provide quite accurate insights, although a thorough understanding requires the complicated analysis of long-run stochastic growth rate.

Correlates of oxidative stress in Soay sheep: A pilot study.

Dan Nussey & Josephine Pemberton.
Jon Blount (Centre for Ecology and Conservation, University of Exeter, Cornwall Campus).

Reactive oxygen species ('ROS') are chemicals produced as the result of a variety of normal activities that occur within our cells. Their build-up can interfere with the normal functioning of cells and tissues and many researchers have implicated ROS accumulation as a driver of the ageing process. Numerous antioxidant chemicals are known to reduce ROS levels, and the balance between ROS production and antioxidant defence is generally referred to as the level of 'oxidative stress'. Growth, reproduction and poor environmental conditions are all likely to be

associated with increases in cellular activity which could lead to heightened levels of ROS and oxidative stress. Differences between individuals' rates of accumulation of ROS may explain differences in life histories or ageing patterns. Remarkably, we currently know next to nothing about either the causes or consequences of variation in oxidative stress in naturally-occurring animal populations.

We used blood plasma samples taken from Soay sheep during the August 2007 catch-up to assay levels of a compound (*malondialdehyde* or 'MDA') which reflects levels of ROS build-up in cell membranes. We were able to show that our MDA assay was not influenced by the amount of time it took for us to process a sample and assays did not vary between catch days or trap used to catch sheep. We were also able to show very high repeatability of MDA assays on the same sample of sheep blood.

Oxidative stress levels, reflected by the amount of MDA in an individual's blood plasma, varied between sheep of different ages (Fig. 7). Lambs of both sexes had higher MDA levels than yearlings or adult females, a result which may reflect the cellular stresses associated with growth in early life. Indeed, the rate of growth of lambs in our sample between birth and capture in August was positively associated with MDA levels. In other words, lambs that grew faster appeared to have higher levels of oxidative stress (Fig. 8).

We had expected to see increased oxidative stress in female sheep that had invested more in reproduction. However, we found no significant difference in MDA levels between yearling females that either did or did not produce a lamb in 2007 and adult female sheep that twinned had lower MDA than females producing singletons. Similarly, females that lost their lambs very early, and thus paid minimal costs of lactating to those lambs, had higher MDA than females with surviving lambs. These results may seem contradictory at first but are very likely to reflect differences in physiological condition or state between females and argue strongly for a more detailed, longitudinal analysis of patterns of oxidative stress across the lifetimes of wild animals. We hope to obtain funding in the near future to extend and develop this pilot study to shed new light on the factors influencing patterns of oxidative stress in the wild animals.

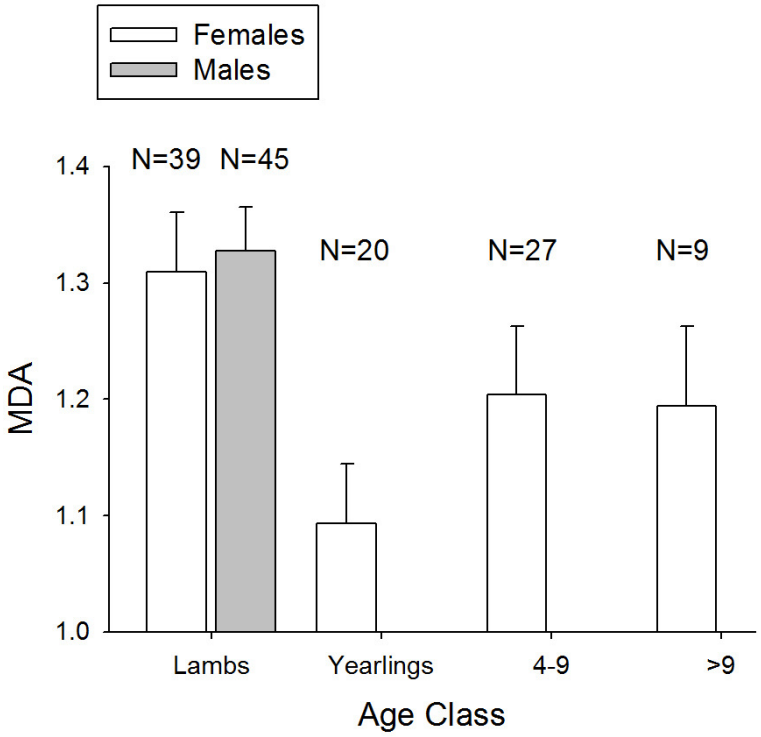

Figure 7. *Differences in mean MDA assay scores between female and male lambs and yearling, adult (4-9 year olds) and senescent (> 9 year olds) females with standard error bars. Sample sizes shown above each bar.*

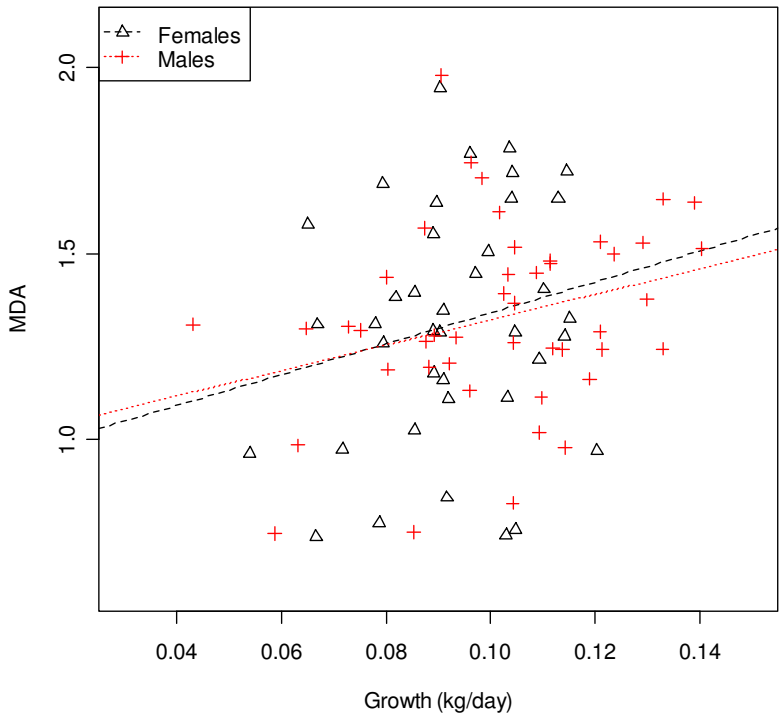

Figure 8. *Plot of MDA assay scores for male (crosses) and female (triangles) lambs against their rate of growth between capture at birth and capture in August in kg per day. The overall trend is significantly positive and there was no difference between the trends within male and female lambs.*

The genetic basis of horn development in Soay sheep.

Susan Johnston and Jon Slate.

The Soay sheep of Hirta, St. Kilda, have an inherited polymorphism for horn type. Males have two horn types: normal (87% of individuals) and scurred (deformed, 13%), whereas females have three horn types: normal (32%), scurred (29%) and polled (hornless, 39%). Previous studies by Matt Robinson and others show that the polymorphism is maintained by opposing selection between the sexes: sexual selection favours large horns in males, but natural selection favours scurred horns in both males and females. Soay horn type is most likely determined by a single locus, *Ho (Horns)*, with three alleles, Ho^{+}, Ho^{L} and Ho^{P} (see Table 2). Dario Beraldi mapped the horns locus to a 16 centiMorgan (cM) region on sheep chromosome 10, but the exact gene responsible has not yet been characterised. Locating the horns locus will improve our understanding of natural and sexual selection acting on the horn polymorphism in Soays.

	Males (N=2348)		Females (N=2189)	
Horn Type	Genotype(s)	Frequency	Genotype(s)	Frequency
Normal	Ho^{+} --- Ho^{L} ---	0.868	$Ho^{+}Ho^{+}$ $Ho^{+}Ho^{L}$	0.322
Scurred	$Ho^{P}Ho^{P}$	0.132	$Ho^{+}Ho^{P}$ $Ho^{L}Ho^{L}$	0.292
Polled			$Ho^{L}Ho^{P}$ $Ho^{P}Ho^{P}$	0.386

Table 2. *Relationships between horn type and proposed Horns locus genotype (as previously proposed by Dave Coltman & Josephine Pemberton), and observed frequencies of horn types of Soay sheep on Hirta.* Ho^{+} *= allele for horns,* Ho^{L} *= allele for sex-linked horns,* Ho^{P} *= allele for polled, --- = any other allele.*

This PhD project continues studies on mapping and selection on horn type in Soay sheep. The aims of the project are to:

1. Fine map the location of the *Ho* locus
2. Understand the genetic control of horn size
3. Examine selection on the horn genotype

Fine mapping the horns locus

Dario Beraldi found a single genetic marker associated with horns (LOD ~ 6.1) with a 95% confidence interval of ~16cM, a chromosome region which potentially contains hundreds of genes. Our first task was to reduce the size of this chromosome region by increasing the resolution of the linkage map for chromosome 10. We used online sheep and cattle DNA sequences to find 23,000 potentially useful microsatellite markers within the sheep genome. We concentrated on the cattle equivalent of sheep chromosome 10 to identify 12 new microsatellite loci (named $OVAR_n$) on sheep chromosome 10. We genotyped these markers in all individuals in the Soay sheep mapping panel and updated Dario Beraldi's linkage map (Fig. 9). Five markers are now associated with horn type, and we have refined the location of *Ho* to a ~7.3cM interval (LOD ~ 7-10).

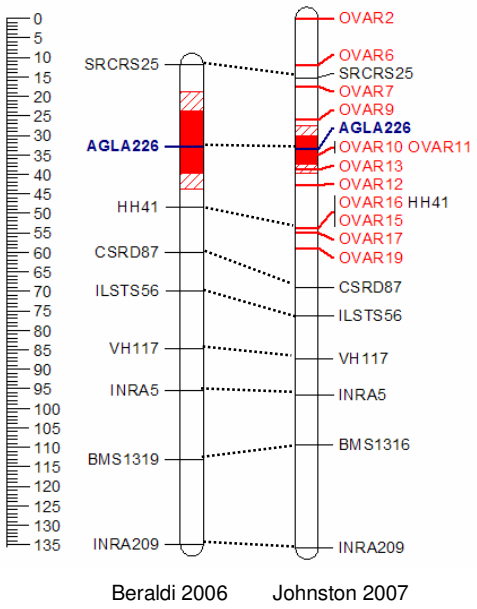

Figure 9. *Linkage maps of Soay sheep chromosome 10 from Dario Beraldi (left) and from the current work (right), showing the location of the Ho locus to confidence intervals of 95% (block shaded) and 99% (diagonal striped).*

At this stage, we still cannot determine individual genotypes at *Ho*. In the equivalent region of the cattle genome, there are around 51 genes, but no obvious candidates for horn development. Future work on fine mapping the Soay *Ho* locus will focus on population-wide associations between further markers and horn type, and then DNA sequencing candidate genes to find the causal mutation for horn type. We then hope to examine the relationship between *Ho* genotype and fitness in the Village Bay Soay population.

Genetic Control of Horn Size

Variation in horn size is both genetic and environmental. We wanted to determine if genotype at the *Ho* locus also affects horn size within horn type. Five of the six possible genotypes at the *Ho* locus give rise to normal horns in males (Table 2). We scanned chromosome 10 for regions of the chromosome associated with variation in normal-horned male horn size, known as quantitative trait loci or QTL. We found a significant QTL for total horn length ($p < 0.01$, $q^2 = 0.268$) and horn base circumference ($p < 0.05$, $q^2 = 0.202$) in normal-horned males (Fig. 10), both of which correspond to the position of the horns locus. This implies that different genotypes at the *Ho* locus result in differences in horn size in normal-horned males.

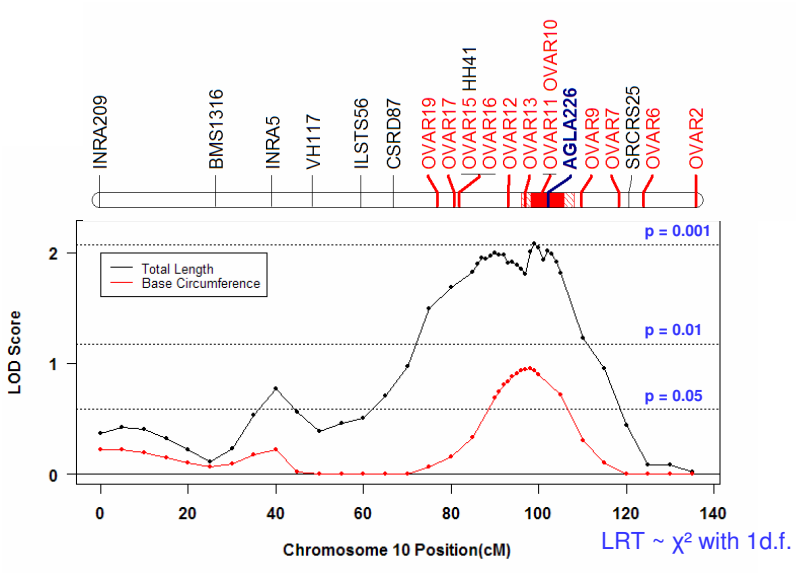

Figure 10. *Map positions of putative QTL for total horn length (upper line) and horn base circumference (lower line) in normal horned males. The LOD score represents the probability of having a QTL in a given position against the probability of no QTL at that position. The top axis shows marker positions on chromosome 10, with the Ho locus represented as in Figure 9. P-values are for a Likelihood Ratio Test distributed as* χ^2 *with 1 degree of freedom.*

The variable environment of St.Kilda generates fluctuating selection on male horn length.

Matt Robinson, Josephine Pemberton and Loeske Kruuk.

The horns of male Soay sheep are used as weapons in competition for mates and larger horns confer increased breeding success. However, allocating resources to horn growth may come at a cost to survival. The relationship between horn growth and a male's lifetime fitness will therefore represent a balance between its relative impacts on breeding success versus viability. Furthermore, because the risk of mortality in the population is determined by ecological conditions, survival costs will vary as a function of the prevailing environment. Because of the variable environmental conditions experienced on St. Kilda, there may not be an optimal level of resource allocation to horn growth. These arguments provide an intuitively appealing explanation for the maintenance of the genetic variation in horn length that we see in the population.

In this study our aim was to assess the genetic architecture of and the selection pressures on male horn length across changing environmental conditions. We did so by examining the relationship (correlation) between male horn growth and three lifetime fitness measures (average fecundity, longevity and lifetime breeding success). The relationship between horn length and fitness (equivalent to the selection pressure on horn length) can be broken down into genetic and environmental components. In this way, we estimated the phenotypic, genetic and environmental associations between horn growth and lifetime fitness in male Soay sheep that experienced different environmental conditions during the year of their birth. The study population is excellent for this purpose as weather conditions, population density, and consequently food availability fluctuate from year-to-year, providing substantial differences between individuals in the environmental quality of their birth year and thus their survival rates. Soay sheep have a distinct

polymorphism for horn development with around 86% of males growing full horns (normal-horned) and 14% growing reduced (scurred) horns. We consider normal-horned males only here, as scurred males do not use their horns to compete for access to mates, and so there is no sexual selection on horn size in this group.

First, we estimated the phenotypic correlations between first year horn growth and fitness for males who experienced different environmental conditions. We used an indirect measure of environmental quality (*E*) of an individual's birth year defined as the proportion of lambs which survived their first winter (proportion surviving ranged from 0.05-0.86, with a mean of 0.41), with low survival indicating a poor environment and high survival indicating a good quality environment. We grouped birth years into four groups (1= very poor conditions; 2=poor conditions; 3=good conditions; 4=very good conditions). Secondly, as phenotypic associations may be environmentally driven as well as having a genetic basis, we extended each model to break down phenotypic associations between first year horn growth and fitness into genetic and environmental correlations. For the genetic associations we determined the genetic relationship between horn length and fitness across environmental conditions ranging from -1 (very poor conditions) to +1 (very good conditions).

We found that the variable ecological conditions experienced by individuals during the first year of their life generated fluctuating selection on horn length (Fig. 11). Phenotypic and genetic associations between male horn growth and lifetime reproductive success (lifetime fitness) were positive under good environmental conditions (due to increased breeding success) and negative under poor environmental conditions (due to reduced survival).

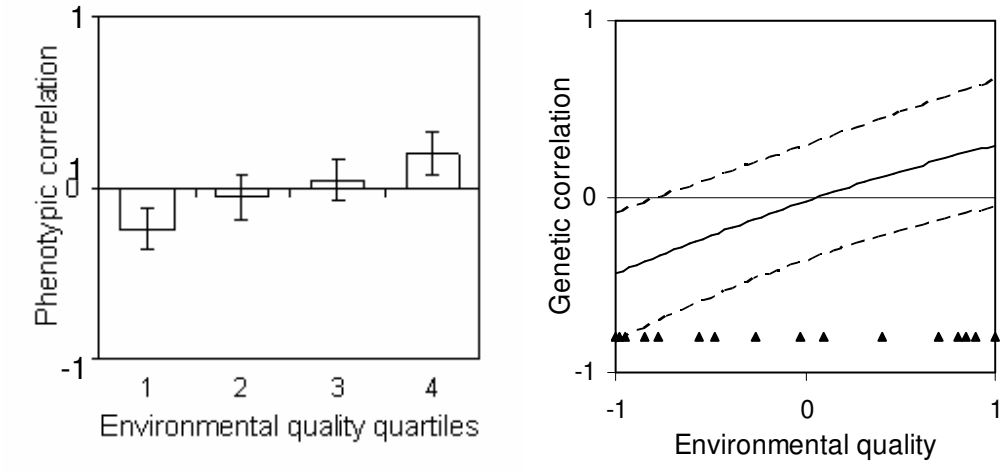

Figure 11. *Phenotypic (left panel) and genetic (right panel) associations between horn length and lifetime breeding success in relation to the environmental conditions experienced by males during the first year of life.*

This means that because of the unpredictable environment, allocating a lot of resources to early horn growth is a gamble which will only pay off if ensuing conditions are favourable. Males seem to be unable to adjust their rate of horn growth to reflect the conditions that they experience and as a result males that grow large horns will be more successful if born into good environments, but are more likely to die early if born into poor environments. Such fluctuating selection may play an important role in the maintenance of genetic variation in horn length.

Body size, fitness and microevolution of coat colour in Soay sheep

Jake Gratten, Alastair Wilson, Allan McRae, Dario Beraldi, Peter Visscher, Josephine Pemberton and Jon Slate.

In Soay sheep, coat colour is either dark brown or light tawny. The polymorphism is determined by a single DNA base change in the *tyrosinase-related protein 1* (*TYRP1*) gene; at this single site, dark sheep have genotype GG or GT and light sheep have genotype TT. From previous studies we know that coat colour is related to fitness because of an association with body size; dark sheep are larger than light sheep and larger size is associated with increased survival and reproductive success. The relationship between coat colour and size is explained by the presence of a quantitative trait locus (QTL) for body size near to *TYRP1* (i.e. they are genetically linked); dark sheep are larger because the G allele (dominant for dark colour) is co-inherited with a QTL allele that confers larger size. Body size is partly genetically determined, and there is evidence for selection for larger size, and for a genetic response to selection. Consequently, dark sheep should show a fitness advantage compared to light sheep and the frequency of dark sheep should be increasing in response to selection for larger size. However, a 20-year time series of phenotypic data shows a significant decrease in the frequency of dark sheep (Fig. 12A).

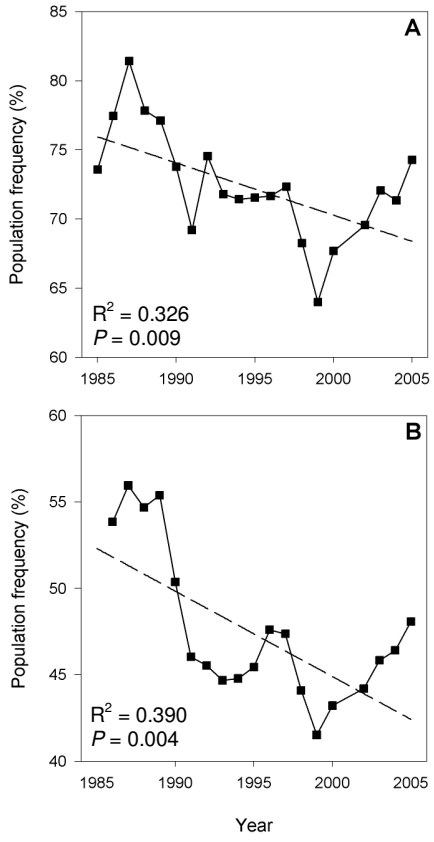

Figure 12. *Estimated frequency of (A) dark sheep and (B) the TYRP1 G allele (dominant for dark colour) in the Village Bay study population from 1985 to 2005. Frequency estimates are from August of each year. Linear regression lines are fitted and show a significant decline in frequency of both dark sheep and the TYRP1 G allele.*

In this report we investigate whether this unexpected trend is due to evolutionary constraints imposed by the presence of other fitness-related genes near *TYRP1*. We compared *TYRP1* genotype for 2509 sheep living between 1985 and 2005 with estimates of lifetime fitness for each indivdiual. We performed parallel analyses to assess the relationship between fitness and coat colour phenotype, data for which was available for the majority of sheep.

We identified a significant genetic association between *TYRP1* and lifetime fitness ($F_{(2,1336)} = 4.03$, $P = 0.020$, $n = 1355$), and showed, using a robust form of linkage analysis that this is due to genetic linkage ($F_{(1,427)} = 6.87$, $P = 0.010$, $n = 492$). Intriguingly, the relationship between coat colour and fitness is inconsistent with that between dark coat colour and large body size. Homozygous dark sheep (GG) exhibited a fitness disadvantage relative to phenotypically identical heterozygous dark sheep (GT) (i.e. a cryptic difference) and light sheep (TT), even though GG sheep are significantly larger than TT sheep (Fig. 13). Conversely, although heterozygous dark sheep (GT) are also significantly larger than light sheep (TT), there was no evidence for differential fitness between these two genotypic classes. At a phenotypic level, the average fitness of dark sheep (genotypes GG and GT combined) was indistinguishable from that of light sheep (TT), and thus there was no evidence for selection on coat colour itself ($F_{(1,2117.8)} = 1.70$, $P = 0.192$, $n = 2334$).

We have demonstrated that the coat colour locus is genetically linked to both body size and fitness. We consider it unlikely that *TYRP1* has direct effects on these traits (a process known as pleiotropy), for two reasons. First, the *TYRP1* protein is only expressed in the skin and eye. As there is no evidence for colour-associated diseases of the skin or eyes in Soay sheep, it is difficult to envisage how *TYRP1* could directly influence size or fitness. Second, none of the *TYRP1* mutations which have been studied in mice or humans have effects on size or fitness. The alternative explanation is that quantitative trait loci for both body size and fitness lie close to *TYRP1* on the same chromosome and are co-inherited with it. Interestingly, there are promising candidate genes for both body size (*VLDLR*) and fitness (*PTPRD*) in the vicinity of *TYRP1* on the equivalent cattle chromosome.

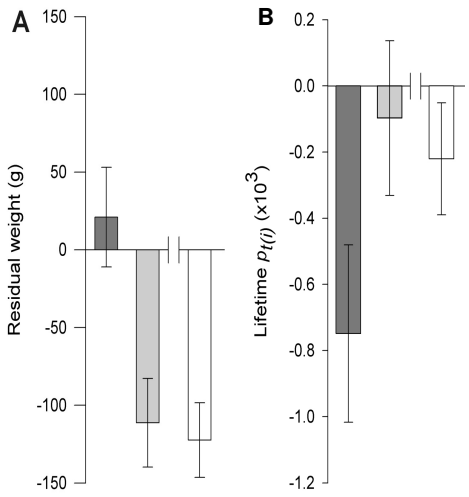

Figure 13. *Bar plots showing the mean (± SE) (A) residual birth weight and (B) lifetime fitness of homozygous (GG) dark sheep (dark grey bars) and homozygous (TT) light sheep (light grey bars), in each case relative to heterozygous (GT) dark sheep (not shown but represented by the zero line), and of light sheep (white bars) relative to dark sheep (not shown but represented by the zero line). Fitness is estimated as lifetime delifed fitness, p_{ti}, a measure which accounts for an individual's relative contribution to population growth.*

Our results therefore imply that the relative fitness of *TYRP1* genotypes is determined by linkage to QTL for both body size and fitness. These QTL appear to have antagonistic effects because the *TYRP1* G allele (dominant for dark coat colour) is associated with large body size (and hence with increased fitness) but also decreased lifetime fitness. Thus, despite an overall positive correlation between body size and fitness, these traits are negatively correlated in the chromosomal vicinity of *TYRP1*. This is because of linkage between the G allele and both a large body size QTL, and a recessive QTL allele with deleterious effects on fitness. Importantly, the direct fitness cost associated with G outweighs the expected benefits of being larger, and this will constrain the frequency of dark sheep. Our results actually imply that the light mutation (T) should be increasing in frequency, although genetic drift will also play a role in determining the allele frequencies. This is consistent with both the observed decrease in the frequency of dark sheep (Fig. 12A), and with a 20-year time series of *TYRP1* genotype data (linear regression, slope of -0.49%/year for frequency of the G allele, $R^2 = 0.390$, $P = 0.004$; Fig. 12B). Thus, the microevolutionary dynamics of coat colour are consistent with expectations arising from the presence of a negative genetic correlation between size and fitness in the vicinity of *TYRP1*.

This study shows that selection acting on or near simple Mendelian traits in natural populations can have a complex genetic basis. This has implications for the study of microevolutionary change in natural populations, because fitness variation at the level of the genotype may not be evident in an analysis of selection on phenotype. Consequently, phenotypic studies may wrongly conclude that selection is not acting on genomic regions containing the loci underlying focal traits, and may be unable to explain the microevolutionary dynamics of trait variation.

Individual variation in recombination rates in Soay sheep.

Jon Slate, Harriet Mellenius, Dario Beraldi & Josephine Pemberton.

Recombination is the term used to describe 'crossing over' of non-sister chromatids during meiosis. The consequence of this process is that genetic material from an individual's parents is exchanged and gametes may contain novel combinations of genetic variants ('alleles'). Recombination is necessary for meiosis to proceed properly, and there is a 50% probability that any chromosome in a gamete is recombinant relative to its parental types. Recombination is important, as it creates new combinations of alleles at linked genes, and so is a mechanism by which genetic diversity is generated. It is widely thought that recombination is the reason for the evolution of sexual reproduction. Although recombination rates are of key interest to evolutionary biologists, they have only rarely been investigated in wild populations.

The Soay sheep genetic linkage map created by Dario Beraldi and described in previous reports was used to map genes underlying phenotypic variation in the Hirta population. A key feature of this (and any) map is that the population average recombination rate between linked genes is estimated and measured in units called centiMorgans (cM), named after the American geneticist TH Morgan, who first described genetic linkage. However, it is also possible to examine whether individuals vary in recombination rate, and if so, to examine the evolutionary genetic consequences of this variation. Here we use the data from the Soay sheep linkage map (588 individuals typed at ~247 microsatellite markers) to examine individual recombination rates.

Does recombination rate vary?

During the map building process, the number of recombination events in the two gametes that formed an individual can be counted. By summing these values across chromosomes, and scaling by the population-wide average an estimate of individual recombination rates can be derived. We first examined whether recombination rates had remained stable temporally (Fig. 14).

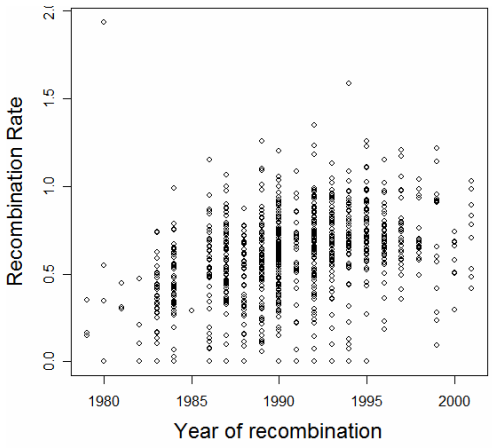

Figure 14. *Recombination rate as a function of time.*

Over the last 20 years of the long-term study mean recombination rate appears to have increased (linear model: Year of recombination: $F_{22,861} = 4.04$, $P << 0.001$). Here each data point represents an individual gamete. Some individuals are represented more than once as they had multiple offspring.

Is recombination rate heritable?
Because individual recombination rates were estimated from related individuals, it was possible to estimate whether recombination rate was heritable, using quantitative genetic techniques. An 'animal model' analysis showed that recombination rate was heritable (0.61 ± 0.04), and this analysis also confirmed the trend that it was increasing temporally (linear mixed effects model: Year of recombination, $F = 2.24$, d.f. = 22, 530.7, $P = 0.003$). The observed heritability is higher than has been reported in humans (e.g. $h^2 = 0.30$, Kong et al 2004, Nature Genetics 36:1203-06).

Is recombination rate under selection?
We investigated whether recombination rate explained variation in fitness. Using both conventional and 'delifed' estimators of lifetime (and annual) fitness we showed consistent patterns for natural selection in favour of low recombination rates (linear mixed effect models, $P < 0.0003$).

Summary
The analyses presented in this report are perhaps the most comprehensive investigation of individual recombination rate variation in a wild population. The current data must be regarded as preliminary, as further investigations into the recombination-rate estimation procedure are ongoing. However, if the findings are robust we have shown that recombination rates have a surprisingly high heritability and are under selection. Intriguingly, selection for low recombination rates is inconsistent with the observation that recombination rate has increased. This example of a counterintuitive evolutionary response to selection will require further investigation.

From the lab to the field: blackface lambs as a model for gene expression analysis in Soay Sheep.

Dario Beraldi, Barbara Craig, Steve Bishop, John Hopkins, Josephine Pemberton.

It is well established that the Soay sheep on St. Kilda are severely infected by gastro-intestinal parasites of which one of the most widespread is the nematode *Teladorsagia circumcincta*. Several studies, therefore, have investigated the characteristics of the parasite population to understand how hosts and parasites interact. Parasitism plays an important role in the population since parasitised sheep are more likely to die in winter and resistance to parasites varies between Soay sheep due to environmental factors, such as being born in a year rich in food supply, but also due to genetic factors. The study of genetic factors is of particular interest since genes can be passed from one generation to another and contribute to evolution. To this end, previous research has attempted to detect which regions of the Soay sheep genetic make-up contribute to the variation in parasite resistance. However, another approach is to determine which genes change in activity or 'expression' during infection. Our project aims at identifying genes that

significantly change expression levels in the presence of infection, and we would like to test whether such genes show genetic variation in the Soay population.

The first part of the project required the characterization of the infection in sheep and it is this part that is presented here. Part of the experimental procedure requires the sacrifice of the animal and since this is not possible in Soay sheep, we chose to study Scottish blackface lambs off the island. The genes found to be differentially expressed in the Scottish blackface lambs will be tested non-invasively in Soay sheep.

Scottish blackface lambs known to be genetically variable for resistance to gastrointestinal nematodes were either exposed to a continuous experimental infection of *T. circumcincta* at the rate expected on St. Kilda (47 lambs) or sham dosed (10 lambs). As a measure of parasitism and host-response, faecal eggs (FEC) were counted over a three month period and *postmortem* burdens measured. The response to infection was also assessed by measuring body weight, IgA antibody level and by counting different white blood cells including eosinophils, which are known to be associated with parasitaemia. Results suggest that the severity of infection was maximal shortly after the beginning of infection when virtually all the flock was infected up to the stage of shedding worm eggs (Fig. 15A). The host response was mediated by increasing IgA antibody levels (Fig. 15B) and eosinophil concentrations. Interestingly, we found that worms hosted in lambs with no detectable faecal eggs (and high levels of IgA) were mostly at the life stage of early arrested larvae (EAL4). In contrast, susceptible lambs (high FEC and low IgA levels) hosted worms mostly at the stage of adults (Fig. 16).

These results should provide a better understanding of the host-parasite interaction and could have applications for the genetic improvement of domestic sheep.

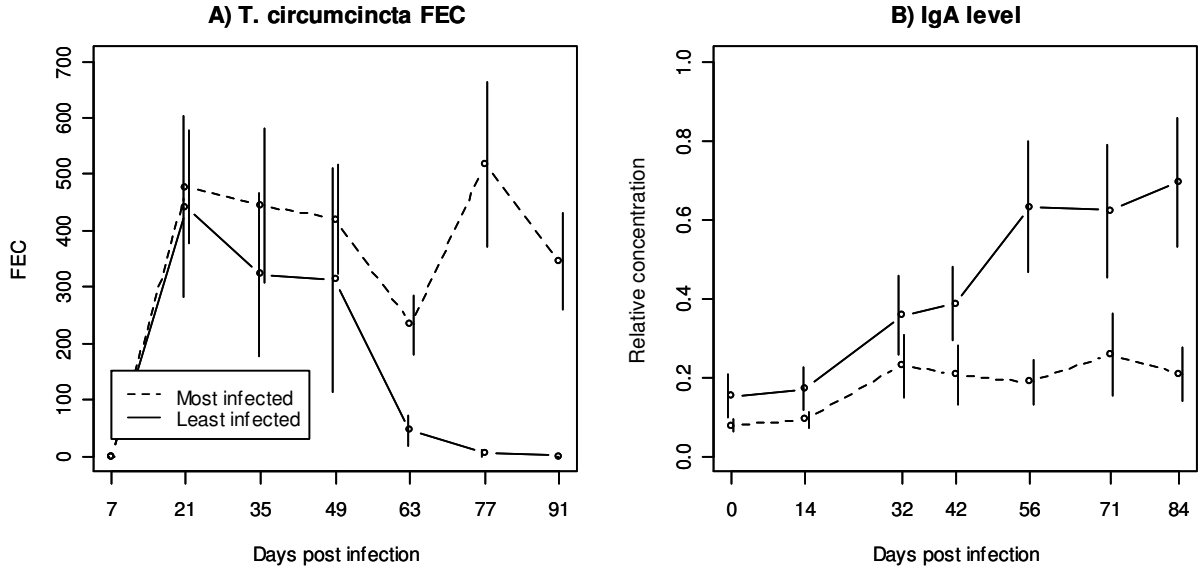

Figure 15. *Comparison of A) Faecal Egg Count (FEC, egg per gram faeces) and B) IgA level (expressed as IgA relative concentration) between the ten most infected Scottish blackface lambs (dashed line) and the ten least infected (solid line).*

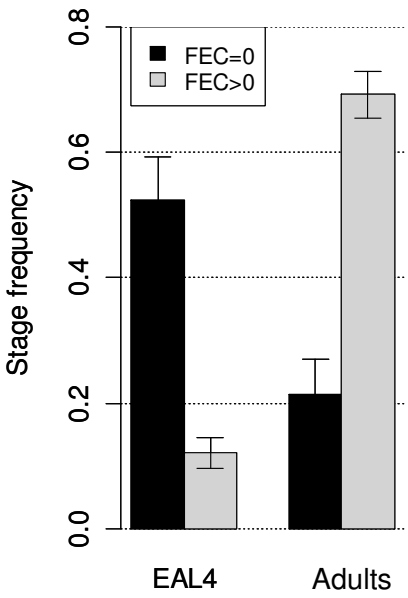

Figure 16. *Post mortem worm burden in lambs with detectable (FEC>0) and undetectable faecal eggs (FEC=0). Error bars are standard errors. EAL4 are arrested larvae and Adults are adult, reproductive worms likely to be shedding eggs.*

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In press:

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APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK

Personnel Change

Adam Hayward arrived in Edinburgh in September 2007 to start a PhD on parasite resistance and ageing. Ana Bento arrived January 2008 at Imperial College and Macaulay Institute to start a PhD on weather-plant-herbivore interactions.

Barbara Craig left Edinburgh for a sheep parasitology position with the Central Science Laboratory, York. Louisa Tempest left Edinburgh to work with the M.O.D., London.

Schedule of work on St Kilda

Winter - Spring

Jill Pilkington monitored mortality during the early part of February and with volunteers, throughout lambing. During this period, detailed data were collected on individual sheep found dead, and samples were taken for genetic and parasitological study.

From March 6th until May 12th, Jill Pilkington, and four volunteers carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 202 lambs were born to 180 ewes; these figures include 22 sets of twins (15 ewes held both lambs, 5 lost one twin). 144 lambs (73 male and 71 female) were caught and tagged; a further 58 lambs died before any tagging attempt.

Summer

Jill Pilkington and two volunteers returned to Hirta on July 13th to carry out ten population censuses, conduct mortality searches (yielding 2 tagged dead animals), and prepare for the main catch-up of study area sheep. The catch-up took place from August 6th – 20th, was led by Josephine Pemberton, and conducted by a team of 11 additional project members and volunteers. 290 sheep were caught and processed, of which 113 were lambs (54 males and 59 females), 31 were yearlings (7 males and 24 females), 13 were adult males, and 133 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 26 Sheep were retagged because of damaged or missing tags. 13 previously untagged lambs, 2 yearlings and 4 adults were caught and processed. Jill Pilkington and two volunteers remained on Hirta until 31st August to complete parasite counts and vegetation monitoring.

Autumn

From October 19th to December 7th Jill Pilkington and two volunteers carried out ten population censuses, monitored the mating period, capturing and processing 35 incoming tups and 10 resident tups. 24 previously darted, non-resident tups were seen in the study area during this rut. Three dead sheep were found.

CIRCULATION LIST - *(Please advise J.Pilkington of any changes or additions)*

Prof. S. Albon	Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Dr. W. Amos	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Ms. S. Bain	NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr. D. Bancroft	GPC AG, Lochhamer Str. 29D-82152, Munich, Germany.
Mr. A. Bennett	NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr D. Beraldi	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. P. Burman	NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. D. Childs	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Prof. T. Clutton-Brock	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Mr. D. Clark	St. Kilda, c/o QinetiQ, Benbecula, HS7 5LA.
Dr. D. Coltman	Dept. Biol. Sci., Univ. Alberta, Edmonton AB, T6G 2E9, Canada.
Dr. T. Coulson	Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. B. Craig	Wildlife, Ecology and Management Group, Central Sc. Lab., York, YO41 1LZ.
Prof. M. Crawley	Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Ms. K. Csillery	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. S. Davies	SNH, Fraser Darling House, 9 Culduthel Road, IV2 4AG.
Dr. A. Drake	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. T. Ezard	Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. J. Fenton	SNH, Great Glen House, Leachkin Rd, Inverness, IV3 8NW.
Ms. J. Ferguson	SNH, Stilligarry, South Uist, HS8 5RS.
Dr. J. Gratten	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Prof. B. Grenfell	Biol. Dept., 208 Mueller Lab., Penn State Univ., PA16802, USA.
Dr. F. Gulland	TMMC, Marin Headlands, Sausalito, CA 94965, USA.
Ms. J. Harden	NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Mr. A. Hayward	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh, EH9 3JT.
Prof. A. Illius	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh, EH9 3JT.
Ms. S. Johnston	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. O. Jones	Dept. Biology, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. C. Klingenberg	Faculty of Life Sciences, Univ. of Manchester, Oxford Rd, M13 9PT.
Dr. L. Kruuk	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh, EH9 3JT.
Dr. G. Lincoln	MRC Centre for Rep. Biol., 49 Little France Cres., Edinburgh, EH3 9EW.
Mr. J. Love	The Watchers Cottage, Snishival, South Uist, HS8 5RW.
Dr. R Luxmoore	NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. A. MacColl	School of Biology, Univ. of Nottingham, NG7 2RD.
Mr. A. Macdonald	18 (1) Cowan Road, Edinburgh EH11 1RQ.
Mr. D. MacLennan	SNH, 17 Frances St., Stornoway. Lewis, Outer Hebrides.
Mr. A. McRae	Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. J. Milner	Hogskolen i Hedmark, Evenstad, NO2480, Koppang, Norway.
Prof. B. Morgan	Inst. Maths.& Stats., Univ. Kent., Canterbury, Kent, CT2 7NF.
Dr. K. Moyes	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Mr. S. Murray	Craigie Dhu, Cardney, Dunkeld, Perthshire, PH8 0EY.
Dr. D. Nussey	Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Dr. S. Paterson	School of Biological Sciences, Univ. of Liverpool, L69 7ZB.
Dr. F. Pelletier	Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Prof. J. Pemberton	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Mrs J. Pilkington	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. B. Preston	Max Planck Inst. Evol. Anthropology, 04103 Leipzig, Germany.
Dr. M Rees	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Mr M. Robinson	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. D. Reuman	Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. J. Slate	Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. R. Stevens	Dept. of Archaeology, University of Cambridge, Downing St., CB2 3ER.
Dr. I. Stevenson	Sunadal Data Solutions, Midlothian, Innovation Centre, Roslin, EH25 9RE.
Dr. G. Sullivan	SNH, SNH, Great Glen House, Leachkin Rd, Inverness, IV3 8NW.
Dr. G. Tavecchia	Imedea-CSIC/UIB, c. M. Marques 21, 07190 – Esporles, Mallorca, Spain.
Dr. L. Tempest	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. P. Visscher	Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. A. Wilson	Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. K. Wilson	Dept. of Biological Sciences, Lancaster University, LA1 4YQ.
Dr. B. Wimmer	Rappstrasse 1, 80687, Munich, Germany.