ST. KILDA SOAY SHEEP & MOUSE PROJECTS: ANNUAL REPORT 2010

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POPULATION OVERVIEW

The sheep population on Hirta entered 2010 at a record level and, as a result, there was some mortality but not a population crash. 117 tagged sheep were found dead within the study area between March and May of 2010. Lambing began on the 31st of March with 73% of lambs born surviving (Fig. 1).

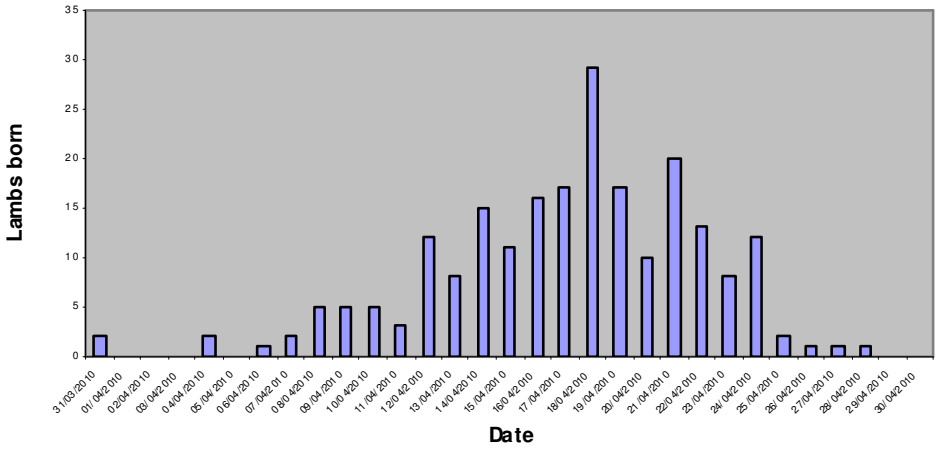

Figure 1. *The temporal distribution of lamb births during 2010.*

In December 2010, 838 tagged sheep were believed to be alive on Hirta, of which 672 regularly used the study area, a total increase of 9% 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.

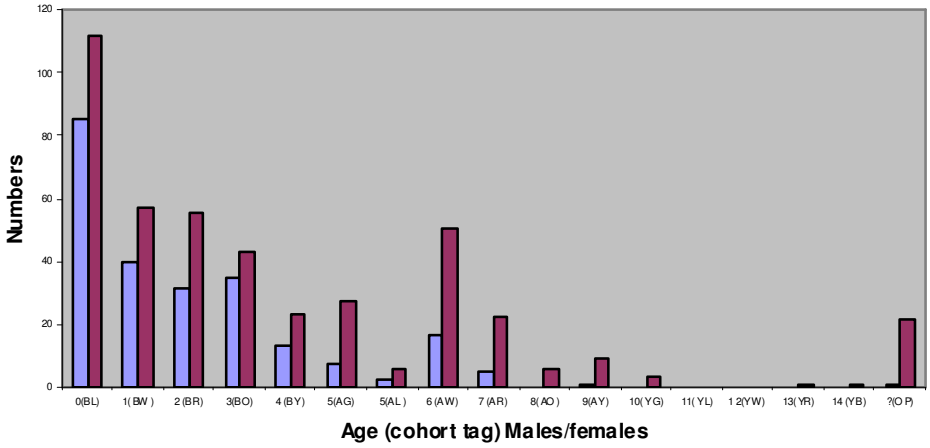

Figure 2. *Age distribution of tagged Soay sheep presumed to be alive at the end of 2010.*

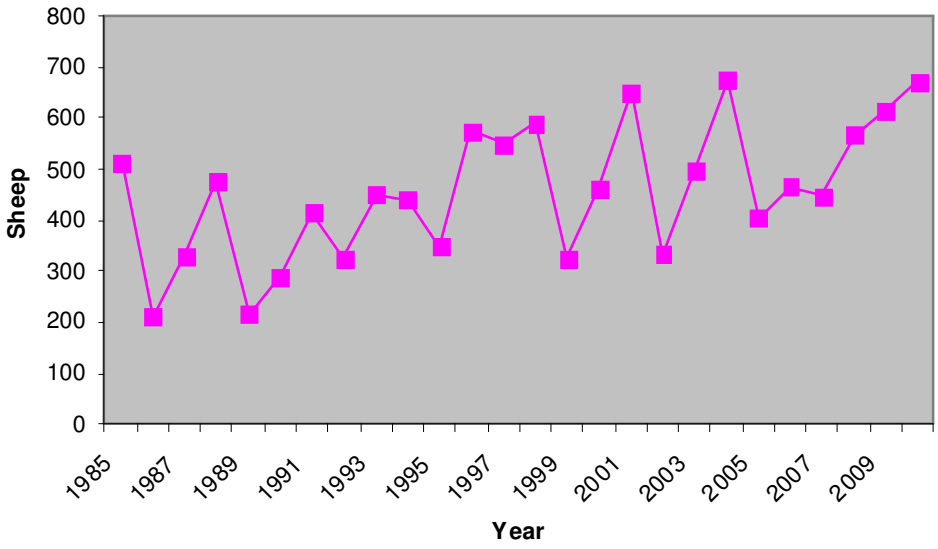

Figure 3. *The number of tagged sheep regularly using the study area since 1985.*

One whole-island count yielded 2058 tagged and untagged sheep, with the details displayed in Table 1. The total population had decreased by 0.7% since summer 2009 when it was at 2208. This gives a delta (calculated as ln (N_{t+1}/N_t)) of -0.07.

Table 1. *Demographic and geographic distribution of sheep observed during the count of Hirta on August 13th 2010. 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	262	8	84	1	98	7	41	0	194	**695**
Mullach Bi/Cambir	264	8	87	2	69	2	27	1	229	**689**
Ruaival/Village	223	10	88	2	100	4	26	0	214	**667**
(Mullach Bi – unclassified sheep)										**7**
Total	**749**	**26**	**259**	**5**	**267**	**13**	**94**	**1**	**637**	**2058**

REPORTS ON COMPONENT STUDIES

Vegetation.
Mick Crawley.

It is now 8 years since our last major population crash, which happened during the winter of 2001-2002. Over this period, sheep numbers have continued their steady increase, as judged by the whole-island count each August, averaging roughly 37 extra animals every year (Fig. 4).

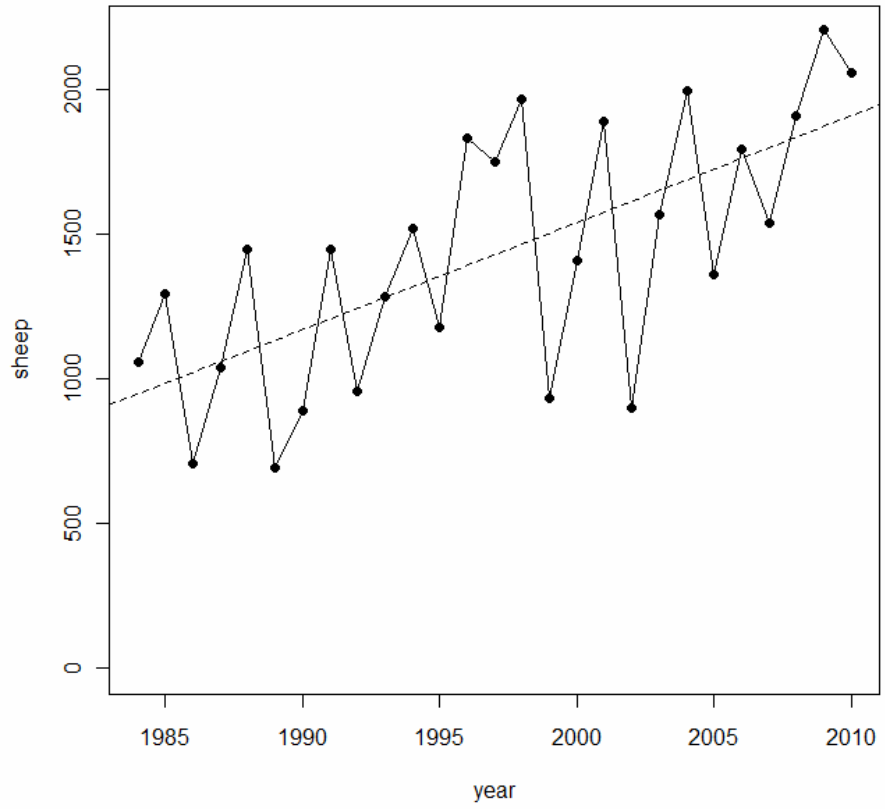

Figure 4. *Total sheep numbers on Hirta from the annual whole-island count each August. The slope of the dotted linear regression line is 36.96 ± 8.20 animals per year. Note that there was a slight decline between 2009 and 2010, but 2010 was still the second-highest population we have ever counted.*

Given that sheep numbers have been food-limited throughout the study period, this raises the question as to how these extra animals are accommodated: is it by a decrease in average plant biomass, by increased plant productivity, or by a combination of both? There has been a significant decline in mean total grass biomass at all seasons of the year, but the decline has been greater in summer biomass than in winter (Fig. 5).

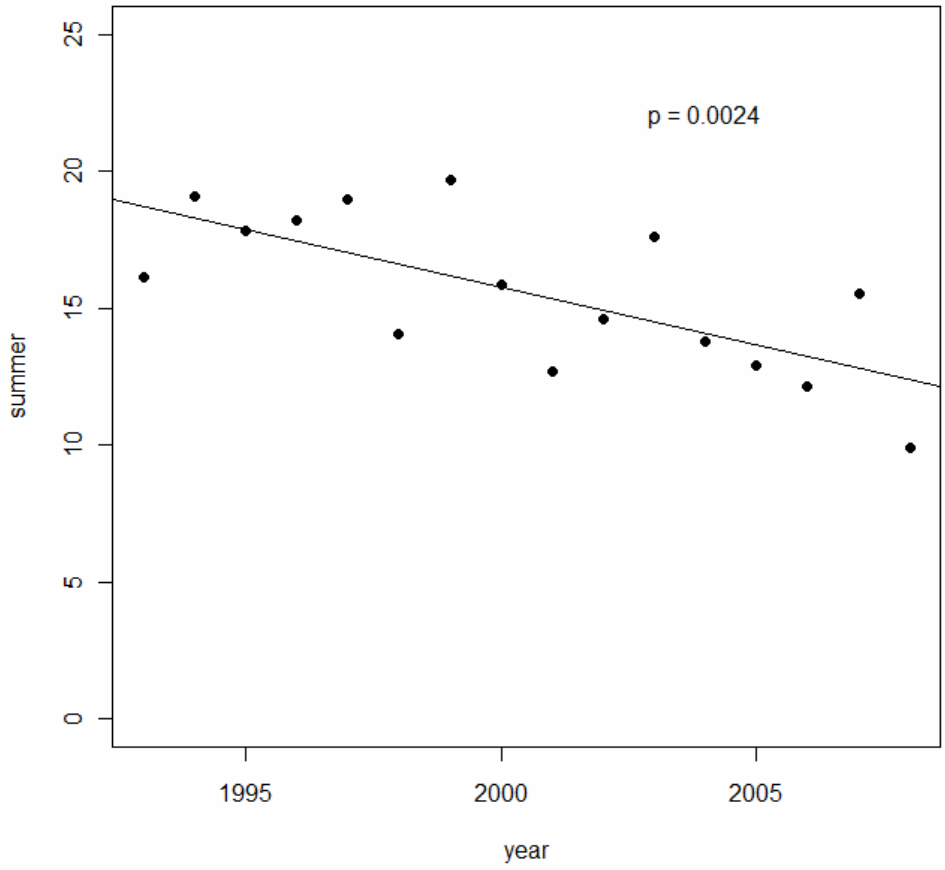

Figure 5. *Mean grass biomass, measured in August on the relatively productive Holcus/Agrostis grasslands inside the Head Dyke, has shown a significant decline as sheep numbers have increased (p = 0.0024). The effect size is a decline of roughly 18% in the standing crop of green biomass at the end of the rapid growth phase (this is the food that has to see the animals through the winter, until rapid grass growth starts again in April). Mean winter biomass is lower, of course, but still declined by roughly 20% over the same period (p = 0.034)*

Productivity is difficult to measure without frequent (e.g. monthly) vegetation samples from temporary sheep-exclosures, but our best information comes from the pyramids (Fig. 6) that we move twice per year (in March and in August).

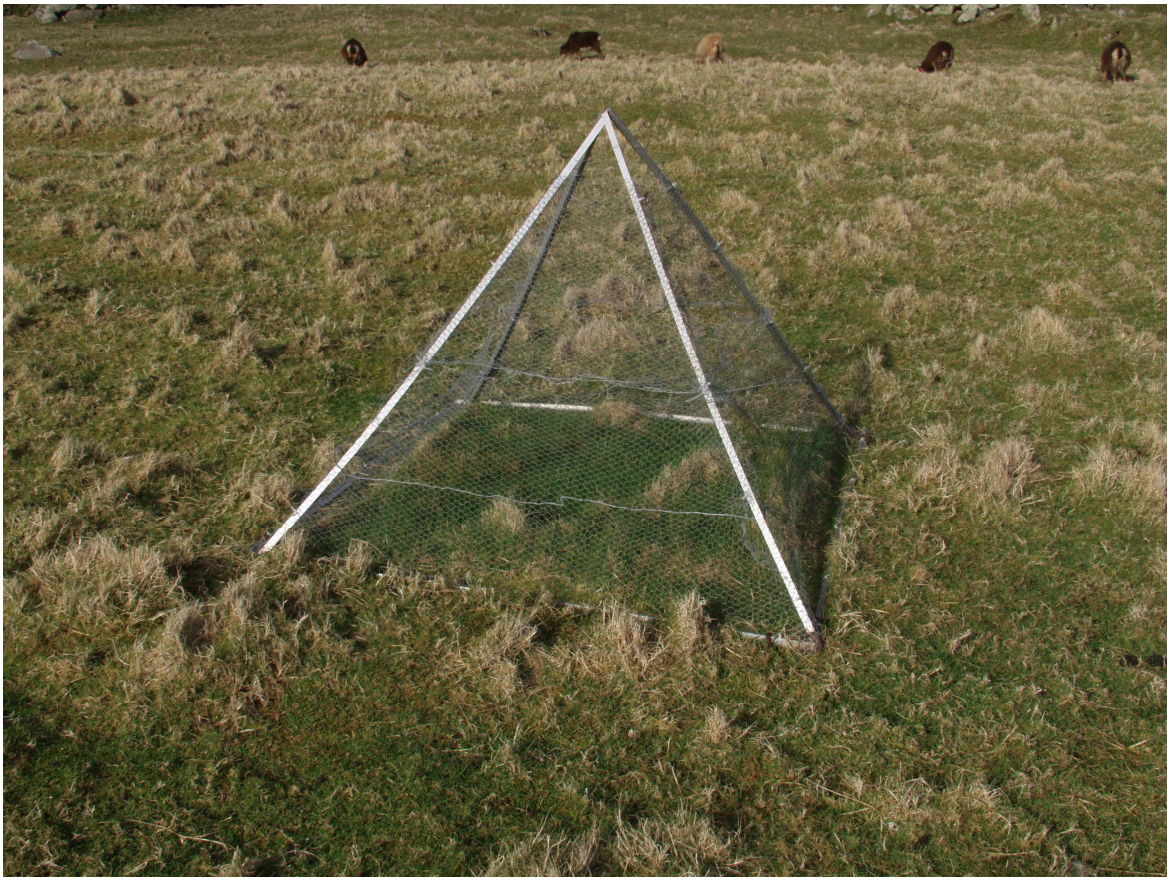

Figure 6. *Temporary grazing exclosures, known as pyramids, shown here in March in Mid Fields on Hirta, are used to estimate grass productivity and grazing offtake by sheep. The extra green biomass inside the pyramid is over-winter grass growth that has been cropped by sheep from the area outside the pyramid.*

The pyramids give us two important numbers per year, representing offtake by sheep in the periods April to August and September to March. As sheep numbers have risen, it is likely that over-winter grass growth has been most consequential in reducing the likelihood of population crashes. The offtake data do not paint a simple picture, however (Fig. 7).

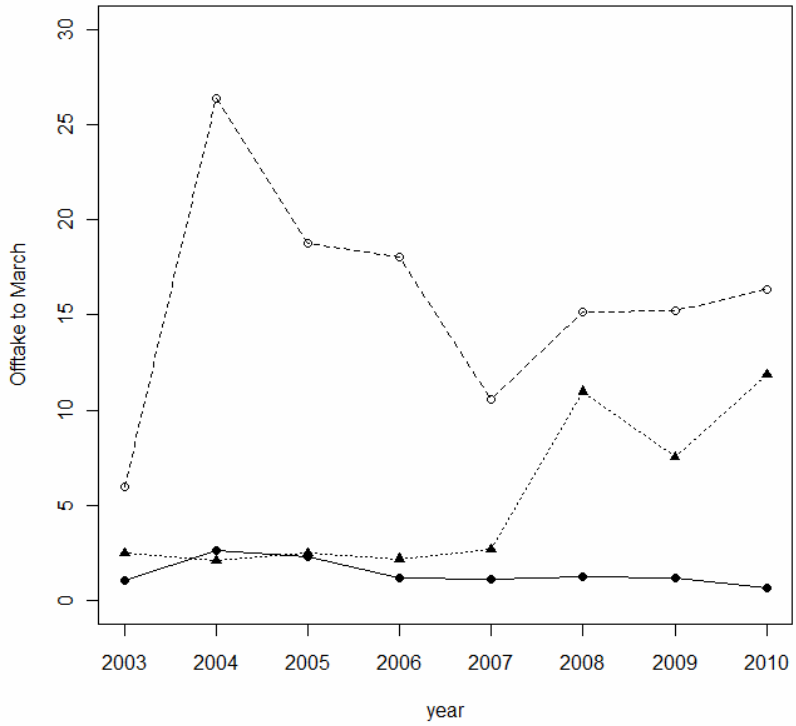

Figure 7. *Offtake, measured as the extra grass biomass inside a pyramid in March following protection from sheep grazing since the end of August, in three contrasting sites within the study area on Hirta. Lower solid line (solid circles) shows very low offtake and no trend in Gun Meadow; middle dotted line (solid triangles) indicates low offtake up to 2007 but a substantial increase from 2008 at St. Columba; upper dashed line (open circles) demonstrates high, but not obviously trended, offtake from the most productive grasslands (the average of West Field and Mid Fields).*

In summary, there is no compelling evidence that increased sheep numbers have been driven by a steady increase in, say, over winter grass growth in all plant communities. There has been a decline in mean biomass and this, in itself, might have had a small positive effect in boosting productivity (by increasing the ratio of young green grass to dead organic matter in the sward). We still have much to learn about how food quantity, food quality and the rate of food production interact to determine sheep demography.

Population dynamics and weather.
Ana Bento.

I have decided on the surrogate dataset for St Kilda and it is going to be Stornoway. The advantages of this are that it provides an unbroken record of daily data covering the entire study period. The downside is that Stornoway has a relatively continental climate when compared with the extreme maritime climate of St. Kilda.

Here I present the first analysis using these data. Table 2 shows the climatic conditions in January, February and March in the years when the population crashed. There is clearly no single climatic cause for all crashes, but, of course, high population density is a pre-requisite along with biased age and sex

structures. What the table shows is that higher than average wind speed in winter is associated with crashes, but none of the other variables shows an absolutely consistent pattern.

Table 2. *Weather variables in the winter months of crash years. The signs reflect a departure from the long-term monthly averages. The '-' indicate values in the 75th percentile and below; the '- -' indicate values in the 90th percentile and below; '0' is somewhere inside the box of a box and whisker plot. The '+' indicate values in the 75th percentile and above. The '++' indicate values in the 90th percentile and above.*

Crash Years	*Month*	*Min. Temp.*	*Max. Temp.*	*Rain*	*Wind*
	January	-	0	-	0
1959/60	February	-	-	+	+
	March	+	+	-	0
	January	+	0	-	+
1966/67	February	++	+	+	++
	March	-	+	++	++
	January	++	++	+	++
1988/89	February	0	+	++	++
	March	-	0	+	+
	January	0	-	+	-
1998/99	February	-	-	+	+
	March	+	+	+	0
	January	++	++	++	+
2001/02	February	-	+	++	0
	March	+	+	-	+

More important is an analysis of the whole time series, rather than just concentrating on crashes. I have taken all the data from the first (1959-1969) and second (1985-present) study periods and fitted a variety of statistical models. In all cases, I began with a complex model containing all weather variables from all winter months and simplified this to obtain a minimum adequate model. A key question is whether the model assumptions about the form of the density dependence have important consequences for the variables retained in the minimal model, their effect sizes and standard errors. To demonstrate this I have contrasted three different models for the density dependence where Delta = log (N_{t+1}/N_t). The models are Delta ~ N, Delta ~ log (N) and Delta ~ StepFunction (N) (See Fig. 8).

In Figure 8a, I show the relationship between the Delta residuals and February minimum temperature, which was one of the variables consistently to appear in the minimum adequate model. This means that warmer Februaries are associated with higher rates of population increase and lower rates of population decline. The remaining three panels illustrate the differences in the form of density dependence across the three models.

In contrast to previous analyses, there is no place for wind or gales in any of the winter months in any of the models. Warmer Decembers are consistently associated with lower values of Delta, as are wetter Januaries (and, in one case out of three, wetter Februaries). The only weather variable positively correlated with Delta was February temperature (See Fig. 8a for minimum temperature, maximum temperature not shown). Readers will be intrigued to note that winter NAO (North Atlantic Oscillation) did not survive model simplification in any of the cases.

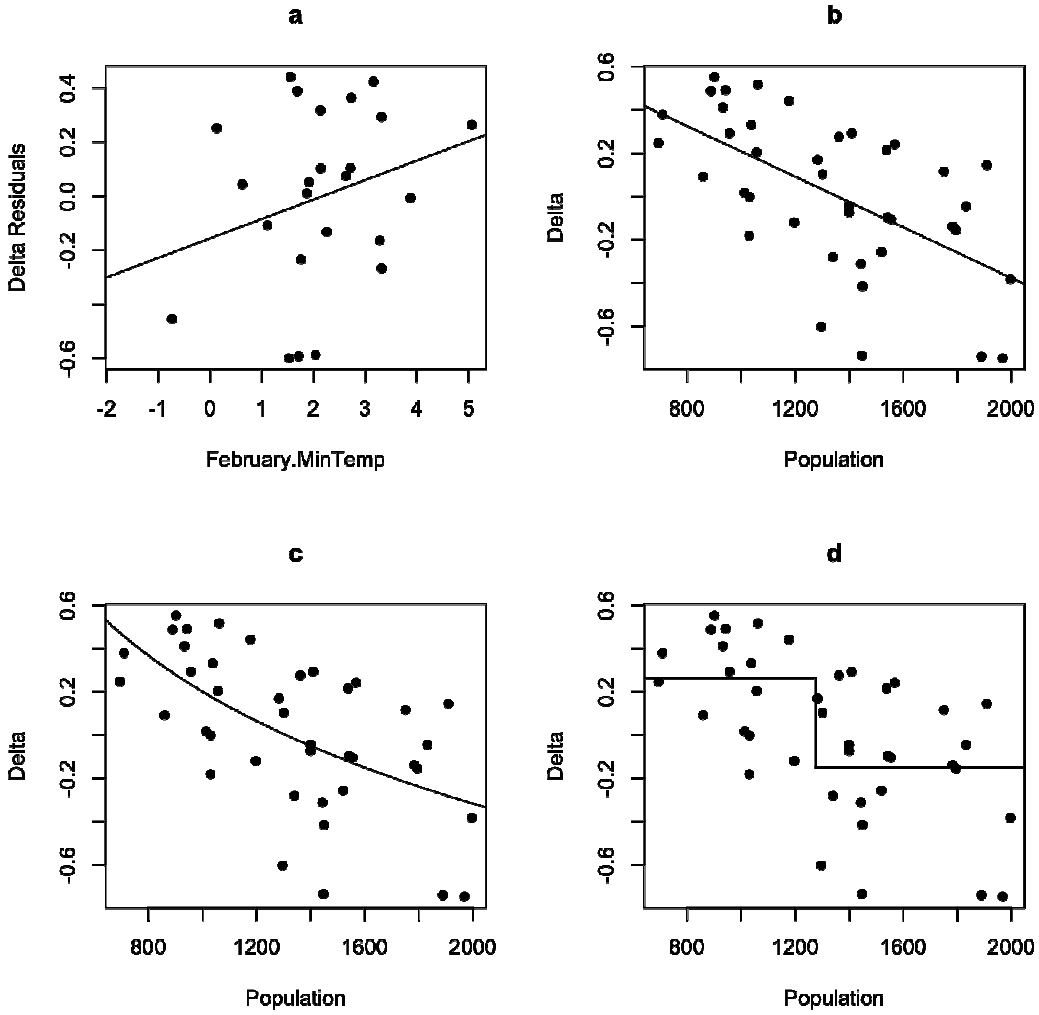

Figure 8. ***a)*** *Shows the significant positive relationship between Delta residuals and February minimum temperature (for details see text).* ***b)*** *Shows the relationship between Delta and population count in August of the previous year.* ***c)*** *Shows the relationship between Delta and log transformed population size.* ***d)*** *Shows the three-parameter step function centred on the threshold population size of 1274 animals. For the models depicted in b, c, and d the weather variables included in the maximal model were Day-degrees (December through mid March with a threshold of 5°C), December minimum temperature, December maximum temperature, December rainfall, December wind speed, January minimum temperature, January maximum temperature, January rainfall, January wind speed, February minimum temperature, February maximum temperature, February rainfall, February wind speed, March minimum temperature, March maximum temperature, March rainfall, March wind speed and the winter NAO (North Atlantic Oscillation).*

Ongoing work is investigating the effects of weather at other times of the year that might affect sheep dynamics through, for instance, effects on plant productivity.

Natural selection on parasite burden: variation across age and environmental conditions.
Adam Hayward, Alastair Wilson, Jill Pilkington, Tim Clutton-Brock, Josephine Pemberton and Loeske Kruuk.

Individuals in natural populations are infected with a range of parasitic organisms, which may reduce their condition and fitness. We therefore expect that natural selection should favour individuals resistant to parasites. However, protective immune responses are energetically costly, and so the best strategy may be to maintain a low level of parasite infection, which neither causes too much damage nor requires a demanding immune response. We also see a vast range of parasite burdens in natural populations. Two interesting questions are therefore i) what is the parasite burden that maximizes host fitness, and ii) what maintains variation in parasite burden? The answer to this second question could be explained by changes in the optimal parasite burden across time- for instance, if high parasite burdens are favoured one year, and low parasites the next, the result could be high diversity in parasite burden as a whole.

The St Kilda Soay sheep are host to a number of parasite species, the most common and abundant of which are the strongyle nematodes, a group of gastrointestinal worms which have negative effects on productivity in domestic sheep. An estimate of an individual's parasite burden is gained by counting the number of eggs per gram of faeces, giving a faecal egg count (FEC). This tends to be very high in lambs, lower in yearlings, and lower still in adults; males have higher counts on average than females, and heavier sheep have lower FEC. Previous experiments on St Kilda have shown that treatment of lambs with anti-parasite drugs increases survival over winter, but results of experiments on adults have been equivocal, with one study finding increased survival in treated females, but with another study finding no effect of treatment on adults at all.

In our study, we estimated natural selection on FEC in all age groups. We tested the effect of FEC on annual fitness, the sum of an individual's survival and reproduction over the course of a year. FEC had a negative effect on fitness in lambs, but not in yearlings or adults. Weight was positively associated with fitness in all sheep, confirming earlier results. We then divided fitness into its two components, survival and reproduction, and we found that lambs with higher FEC were less likely to survive, confirming earlier experimental work. We also found that female lambs with higher FEC were less likely to rear offspring the following year, and that this was due to inability to raise the lamb from birth to independence (the probability that a lamb became pregnant was unaffected by FEC). This suggests that parasite burden does not affect reproductive physiology or developmental rate, but that instead young females with high parasite burden use energetic resources to fight infection rather than to produce a vigorous lamb. Finally, we found that negative selection in lambs only occurred during years of low mortality. In these years, sheep with higher FEC had lower fitness, but in years of high mortality, all lambs had low fitness that was not associated with FEC (Fig. 9).

Our results show that there is negative selection on FEC in the St Kilda Soay sheep population. However, it also shows that this only occurs in lambs, and even then only in certain environmental conditions. This suggests a potential mechanism for the maintenance of variation in FEC in this population. Other mechanisms include the genetic complexity of parasite resistance, the large number of different parasite species infecting the sheep, and the fact that parasite burden is correlated with condition, which is another complex trait. Previous and ongoing work has shown that sheep immune responsiveness may also affect survival over winter - by incorporating immunology into studies of parasite burden we will gain a better understanding of selection on parasite resistance in natural populations.

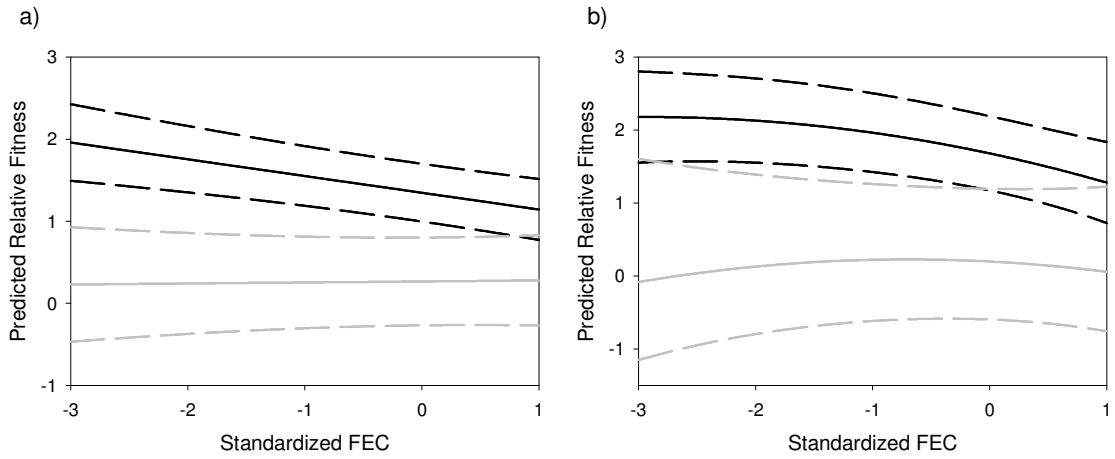

Figure 9. *The relationship between parasite burden (standardized FEC) and annual fitness in* ***(a)*** *female lambs and* ***(b)*** *male lambs. In both cases, FEC is negatively associated with relative annual fitness in non-crash years (black lines), but is not associated with fitness in crash years (grey lines). Solid lines indicate model predictions; dashed lines indicate 95% confidence intervals.*

Identifying parasite resistance genes in Soay sheep and in other breeds.
Emily Brown and Jon Slate.

Individuals and populations are constantly challenged by parasites, which act as one of the main factors influencing survival and reproduction. However, despite the obvious benefit of fending off disease, hosts remain susceptible and immune responses vary widely across species and situations. Much work has focused on trying to understand why this variation in resistance exists.

For sheep and other domestic ruminants, gastrointestinal nematodes are one of the most important classes of parasite. Resistance to these parasites in sheep has been shown to have a genetic basis; in both domestic sheep and Soays resistance is moderately heritable, ranging from approximately 10% to 50%.

Whilst we know that there is genetic variation underlying resistance, we do not know specifically what genes are involved. The aim of my work is to identify genes involved in resistance to *Teladorsagia circumcincta* (the predominant nematode parasite) in Soay sheep, and also to examine whether these genes are relevant in other sheep breeds. Hence, we are interested in asking the question of whether there is a common set of genes that explain variation in parasite resistance both within and between breeds.

Knowing what genes are involved in parasite resistance means that we can then address the question of why or how genetic variation in resistance is maintained. One way of doing this is to look at associations between an individual's genotype and their lifetime fitness. One theory of how genetic variation is maintained is that genes underlying variation in one trait have adverse effects on other fitness-related traits, such that the independent evolution of a single trait will be constrained. This phenomenon is termed antagonistic pleiotropy by evolutionary geneticists.

My work so far has involved two separate analyses. The first involved carrying out DNA sequencing in order to find variation in candidate immune genes in Soay sheep. The second has involved looking at inter-breed variation in resistance to gastrointestinal nematodes, and subsequently examining whether or how this variation is explained by genetic differentiation between breeds.

Analysis 1: Soay sheep immune genes.
The aim of this work is to identify candidate immune genes in Soay sheep, and to sequence them to find genetic variants called single nucleotide polymorphisms (SNPs) within these genes. This was done by carrying out a new genomics approach that enables researchers to determine the DNA sequence of many genes very rapidly. Until recently, the search for genetic variants was laborious because each gene had to be sequenced one at a time. The new approach, termed a NimbleGen experiment, allows researchers to specify the regions of the genome they are interested in, and capture these regions on a custom made chip. The target sample is then sequenced using a so-called next generation sequencer, which is capable of deciphering billions of bases of DNA in a short time.

To carry out this experiment, it was first necessary to compile a list of Soay sheep candidate immune genes. There are some obvious candidates from the Soay sheep literature, for example the Major Histocompatability Complex and the Interferon-Gamma locus. However, there are likely to be many more genes involved in resistance to gastrointestinal nematodes. To compile a list of candidate genes, a variety of resources were used, including data from studies carried out using both Soay sheep and other breeds, such as Blackface sheep. In total the Nimblegen chip was designed to capture 104 candidate genes, and also 49 random genes. These random genes were included as controls, so that variation seen at immune genes could be compared to that found at the random genes.

Soay DNA was pooled into one sample of twenty individuals with high resistance to *T. circumcincta* (low faecal egg count, FEC), and one sample of 20 individuals with low resistance (high FEC). Sequencing was carried out by the University of Liverpool, and five hundred million base pairs of sequence data was returned in December 2009.

Using this data 6567 SNPs were identified; at each SNP there can be two possible bases (or alleles), for example, either an A or a G. For each SNP the frequency with which the different alleles occurred in the two different pools of sheep was calculated, and Fisher's exact tests were used to test for significant differences between the two pools. Hence, we were looking for any differences that could be associated with being more or less resistant to gastrointestinal nematodes.

344 SNPs with significantly different allele frequencies between the pools were found ($P<0.05$). This number represents 5.2% of the total number of SNPs identified, which is no more than you would expect by chance, suggesting that these differences may not be related to resistance. There was also no significant difference between the number of SNPs with significantly different allele counts in candidate immune genes and in random genes ($P = 0.77$) (see Table 3.), showing that we were not more likely to find significantly different SNPs in immune genes.

Table 3. *The number of SNPs found in candidate immune genes and random genes that were either significant at $P < 0.05$, or not significant. The proportion of SNPs within the different categories of gene that were significant is also reported.*

	Candidate immune genes	Random genes
Significant at $P < 0.05$	226	116
Not significant at $P < 0.05$	4388	1750
Proportion significant	0.05	0.06

Analysis 2: Other sheep breeds.
The NimbleGen experiment identified differentiated SNPs in Soays, and although these differences may not be related to resisitance, it is still possible to try to address the question of whether there is a common set of genes that explain variation in resistance within and between breeds. To examine variation in resistance between breeds, data made available by the International Sheep Genomics Consortium (ISGC) project were used.

This project has made a great deal of genetic data (49,000 SNPs) available for a number of different sheep breeds from all over the globe. However, to do this analysis it was first necessary to work out which breeds are more or less resistance to gastrointestinal nematodes. A number of studies have been carried out that measure FEC of sheep, but comparing these studies is difficult, as many factors vary between them; for example the age of the sheep used and a number of experimental details such as whether sheep were left to graze naturally or were artificially infected with parasites. Using a statistical model which accounts for these different factors, breed-specific average FECs could be calculated.

Sufficient information was available to carry out this analysis for 17 breeds, which were also included in the ISGC project: African Dorper, Ethiopian Menz, Red Maasai, Australian Coopworth, Australian Polled Dorset, New Zealand Romney, Barbados Blackbelly, Rambouillet, Merino, Suffolk, Texel, Gulf Coast Native, Merinolandschaf, Scottish Blackface, Soay, Santa Ines and Sumatran. Based on their average FECs, these breeds were separated into two populations, either resistant or susceptible.

Using the genetic data available for these breeds, an F_{ST} analysis was then carried out (Fig. 10). F_{ST} is a measure of genetic differentiation, and is used to identify loci that are under selection. These loci are known as 'outlier loci', and are loci that have excessively high or low F_{ST} compared to neutral expectations. Because we divided our populations based on how resistant the different breeds were, we can assume that any differences in the level of genetic differentiation are related to resistance.

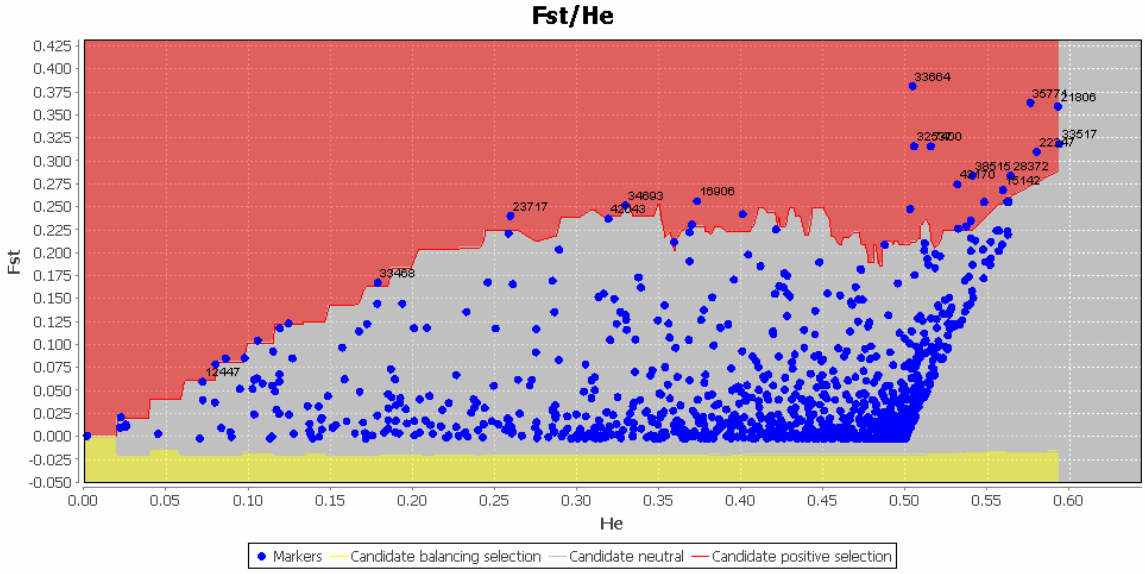

Figure 10. *An example output from Lositan, the programme used to carry out the F_{ST} analysis. Loci falling into the section coloured in red (the top portion of the figure) are outlier loci with excessively high F_{ST}.*

205 outlier SNPs that had excessively high F_{ST} were identified, which is only 0.41% of the total SNPs examined. Nevertheless, we decided to examine whether it was possible to find immune genes near these SNPs. We also examined 205 neutral SNPs, which did not have excessively high or low F_{ST}s, and found that there were more outlier SNPs closely linked to immune genes than neutral SNPs ($P = 3.813\ e^{-06}$). In order to see whether there was any overlap between the two analyses, we also looked at the distance of the outlier SNPs from the significant SNPs from the NimbleGen experiment. Only 11 of the outlier SNPs were within 1 million bp of the significant NimbleGen SNPs, and there was no significant difference in the number of outlier and neutral SNPs within 1 million bp of the NimbleGen SNPs.

In conclusion, when examining SNPs in two groups of Soay sheep that are either resistant or susceptible to gastrointestinal nematodes, we did not find more significantly different SNPs than expected by chance. Significant SNPs were also no more likely to be found in candidate immune genes than in random genes. This suggests that, in our case, the candidate gene approach has not been very successful in identifying immune genes. In contrast, when using the F_{ST} approach and examining genetic differentiation between a number of different sheep breeds, we did find evidence to suggest that outlier loci are related to immunity. However, only a few of these SNPs were found within the same region as the significant SNPs identified in Soays. This suggests that we have not found a common set of genes explaining variation within and between breeds.

A final test of whether the loci that we have identified in the two analyses are really related to resistance or not, is to type the most highly differentiated SNPs in a larger number of Soay sheep. By doing this we can examine whether these SNPs explain variation in parasite burden (FEC) and other immune measures (see item from Andrea Graham and colleagues else where in this report). In order to examine whether there is any evidence of trade-offs that could be constraining the evolution of resistance, associations between SNPs and fitness will also be examined.

By the next annual report, we should know whether our best candidate SNPs can tell us something about parasite burdens and immunity or not.

Immunoheterogeneity and immunosenescence in Soay sheep.

Andrea Graham, Dan Nussey, Kathryn Watt, Adam Hayward, Tom McNeilly, Rose Zamoyska, Jacqui Matthews, Al Nisbet, Kathleen Morriss, Josephine Pemberton, Jill Pilkington.

In the 2009 report we wrote about collaborative work investigating the causes and consequences of variation in a marker of autoimmunity in the Soay sheep population which was published in 2010. Over the last 12 months or so, we have been working to develop new immunological markers and address new questions relating to the variation in immune responses between individuals (immunoheterogeneity) and patterns of declining immune function with age (immunosenescence). Here we provide an update on this work.

Immunoheterogeneity: Our previous work on the Soay sheep included the somewhat counterintuitive finding that strong autoimmune antibody responses were associated with long lifespan. This suggests that self-reactivity doesn't necessarily cause disease and may instead be a side-effect of potent, life-prolonging immune responses to infection. We are therefore investigating immune responses directed against intestinal nematodes that are prevalent among the sheep. In collaboration with Jacqui Matthews and Al Nisbet (Moredun Research Institute), we began by measuring concentrations of antibodies specific to *Teladorsagia circumcincta* in the same August catch-up samples in which we already measured autoimmune antibodies. Adam Hayward is currently testing whether nematode-specific and

autoimmune antibodies are positively correlated and how they relate to nematode faecal egg counts. Thus far, we have found that nematode-specific and autoimmune antibodies are indeed positively correlated overall (Fig. 11). Interestingly, it appears that in lambs the strength of the relationship depends upon nematode burden. With Kathleen Morriss (Princeton University), we measured *T. circumcincta*-specific antibody in plasma samples taken from newborn lambs to investigate maternal transfer of antibody to neonates. If we detect such transfer and can identify its effects on lamb health and survival, this work may help understand why parasites have such variable impacts on lambs.

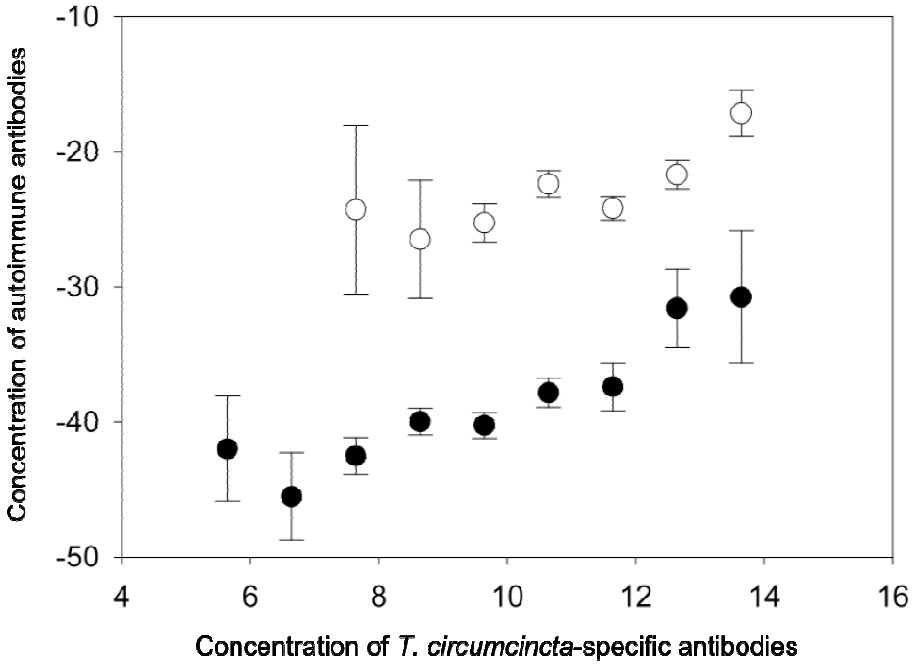

Figure 11. *The relative concentrations of autoimmune versus Teladorsagia circumcincta-specific antibodies measured in plasma collected during 1997-2007 August catch-ups. In both lambs (filled symbols) and ewes (open symbols), there is a significant positive correlation between concentrations of the two types of antibody.*

Immunosenescence: In humans, immune function declines in a complex manner in later life and this deterioration may play an important role in the health problems associated with old age. Studies testing for declines in immune function with age in wild animals remain rare and mostly focus on rather different markers than are used in immunological studies of humans and lab mice. In collaboration with Tom McNeilly (Moredun Research Institute) and Rose Zamoyska (Institute of Infection & Immunology Research, University of Edinburgh), we collected blood samples during the August 2010 catch-up on St Kilda with the aim of testing for ageing patterns using well established markers of immunosenescence. For instance, we looked at how the proportions of naïve immune cells (which have yet to become activated by binding to a particular pathogen / parasite) versus activated cells changed with age in ewes. Studies in humans and lab mice clearly demonstrate that the naïve proportion declines with age as the organ (the thymus) that produces naïve cells deteriorates. Our analyses reveal a similar and dramatic decline in Soay sheep as well (Fig. 12), which we think is the first evidence of its kind from a wild mammal. In future, we hope to collect more samples to investigate the causes and consequences of variation in proportions of different immune cell types and we are currently developing assays of inflammation which we will also use to understand variation in immune responsiveness and immunosenescence in Soay sheep on St Kilda.

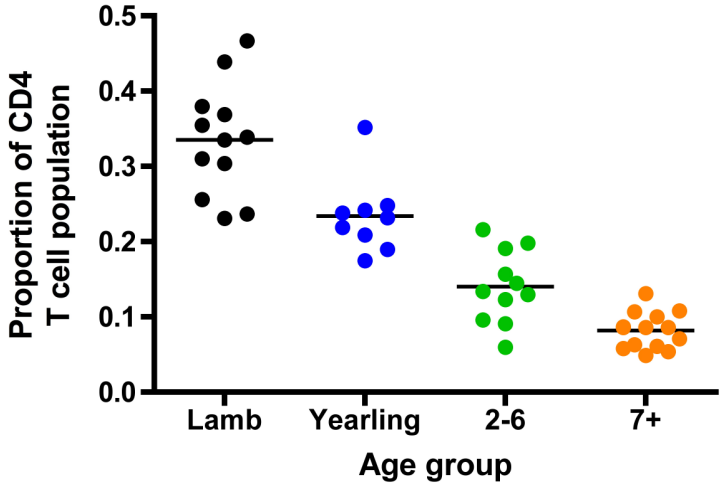

Figure 12. *The proportion of T helper immune cells which were in a 'naïve' (as opposed to activated) state across four age classes in Soay ewes caught in August 2010. The bars are medians for each age group; all differences between age groups were statistically significant.*

The evolution of horn size and horn shape in Soay sheep.
Susan Johnston and Jon Slate.

In sheep, horns play an important role in the competition between males for access to females during the mating season. Soay sheep are unusual in that they have inherited differences in horn type in both males and females, controlled by a single hypothetical locus, *Horns*. The majority of males have large, normal horns, whereas a small number of males (~12%) develop vestigial horns, known as scurs, which cannot be used in competition for mates. Females have smaller horns and are either normal-horned (33%), scurred (39%) or polled (hornless; 28%). It is unusual that these differences persist in the population, as scurred males have fewer offspring compared to normal males, and polled females have fewer offspring and reduced survival compared to normal and scurred females. Therefore, the aims of our study were to find the *Horns* locus and to investigate the relationship between *Horns* genotype, reproduction and survival. This will allow us to understand the evolution of differences in horn type within the population.

Mapping the *Horns* locus.
We examined the association between ~36,000 mutations in the genome (called single nucleotide polymorphisms, or SNPs) and found a strong association with SNPs adjacent to the gene *RXFP2* on chromosome 10 (Fig. 13), a gene which is known to determine primary sex characters in humans and mice. We examined 21 additional SNPs in and around *RXFP2*, developed by colleagues at AgResearch Ltd. New Zealand, and identified a single SNP which was strongly associated with horn type in both males and females (SNP10; Fig. 14). Evidence from SNP10 indicates that there are two forms (alleles) of *Horns* gene in Soay sheep, **+** and **P**. In males, *Horns* is likely to be a dominant locus, where **++** and **+P** males develop normal horns, but **PP** males develop normal horns and scurred horns in a ratio of approximately 1:1. In females, *Horns* is likely to be an additive gene, where the genotypes **++**, **+P** and **PP** confer normal, scurred and polled phenotypes respectively. The *Horns* locus also affects horn size in

normal-horned males; having more copies of + leads to the development of larger horns (Fig. 15). We predict that levels of expression of the candidate gene, *RXFP2*, may explain most, but not all of an individual's likelihood of developing a particular horn type.

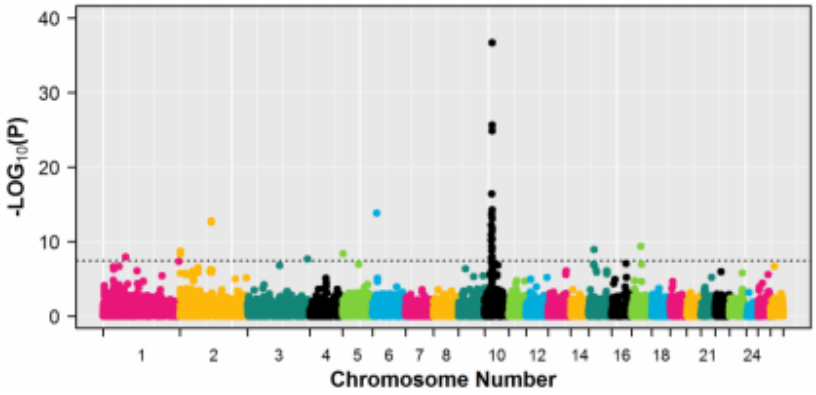

Figure 13. Genome-wide association for horn type in Soay sheep. *Each point represents the association between a SNP and horn type in all sheep. Dotted line = 99.9% confidence threshold.*

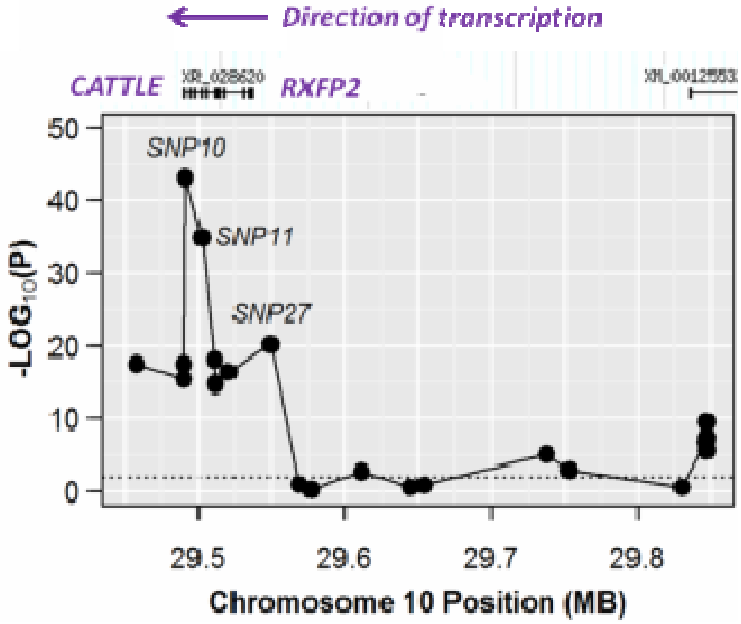

Figure 14. Association between horn type and SNPs around RXFP2. *The position of RXFP2 (the candidate locus for Horns) is shown above the plot.*

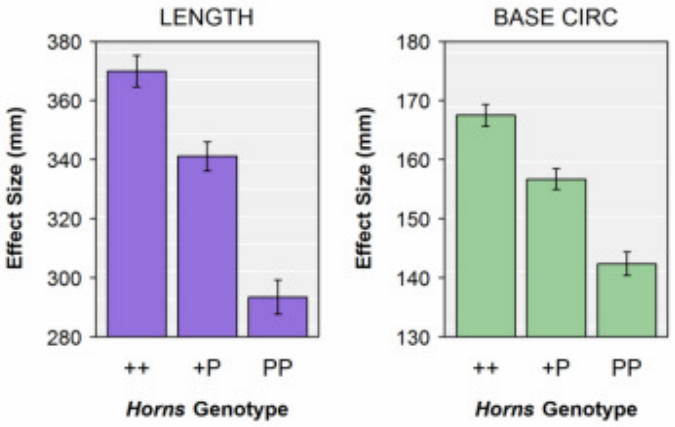

Figure 15. The effect of each horns genotype on horn length and horn base circumference in normal-horned males. *Effect sizes were estimated using an animal model.*

A maternal effect on horn type in sons.

One interesting finding is that around half of **PP** males develop normal horns, with the remainder developing scurs. As male horn type is determined very early in life, we examined the effect of maternal horn type (as an indicator of *Horns* genotype) on horn development in male offspring. We found that **PP** males were 1.72 times more likely to develop scurs if their mother was polled (i.e. it is likely that she does not carry the **+** copy of the gene). We also examined the effect of maternal horn type on horn size in male lambs, taking into account their own individual *Horns* genotype; male lambs with polled mothers developed significantly smaller horns than those of normal mothers (Fig. 16). Therefore, maternal expression of *RXFP2* may also contribute to the likelihood of her sons developing either normal or scurred horns.

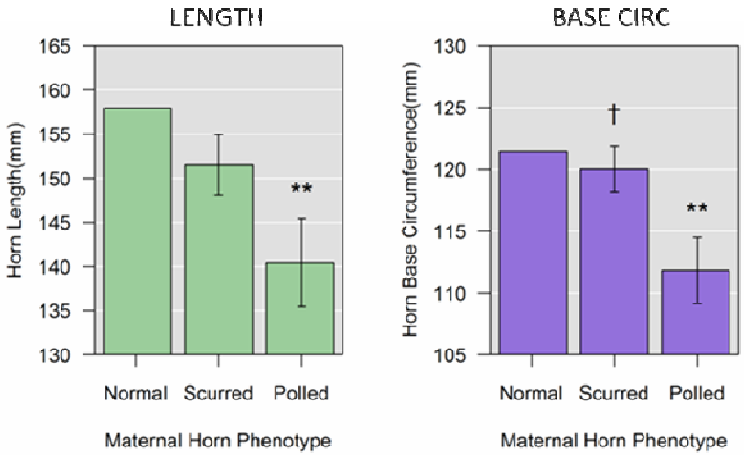

Figure 16. Mean horn size measures in normal-horned male lambs within each maternal horn type. *Mean values and standard errors were estimated from general linear models including offspring Horns genotype as an additional fixed effect; standard errors are given relative to the intercept (normal horns). ** = 99% confidence that the mean is significantly different from that of normal horns. † = 95% confidence that the mean is significantly different from that of polled phenotype.*

Heterozygote advantage at the *Horns* locus maintains differences in horn type.
Another interesting finding was that the frequency of the **P** allele is increasing, even though carrying both copies of **P** can lead to scurred horns in males and the polled phenotype in females. To find out why, we examined the relationship between the genotype at *Horns* and their contribution to population growth, a combined measure of their reproductive success and survival. This allowed us to examine if heterozygote sheep (i.e. those carrying copies of both **+** and **P**) had a selective advantage, which would result in the maintenance or increase of the **P** allele in the population. We found that on a yearly basis, **+P** males had a significantly greater contribution to population growth than **++** and **PP** males (Fig. 17). Over their lifetime, **++** and **+P** males had a significantly greater contribution to population growth than **PP** males, and **+P** females had a significantly greater contribution than **++** and **PP** females (Fig. 17). Therefore, it appears that the *Horns* locus is under selection in this population. As *Horns* controls horn size as well as horn type, the action of this gene may make individual regulation of horn growth difficult. Therefore, one explanation for heterozygote advantage may be that larger horns confer an advantage in male competition, favouring the **+** allele, but limited horn growth may be advantageous in poor environmental conditions, favouring the **P** allele, resulting in a selective advantage for sheep carrying both copies of the gene.

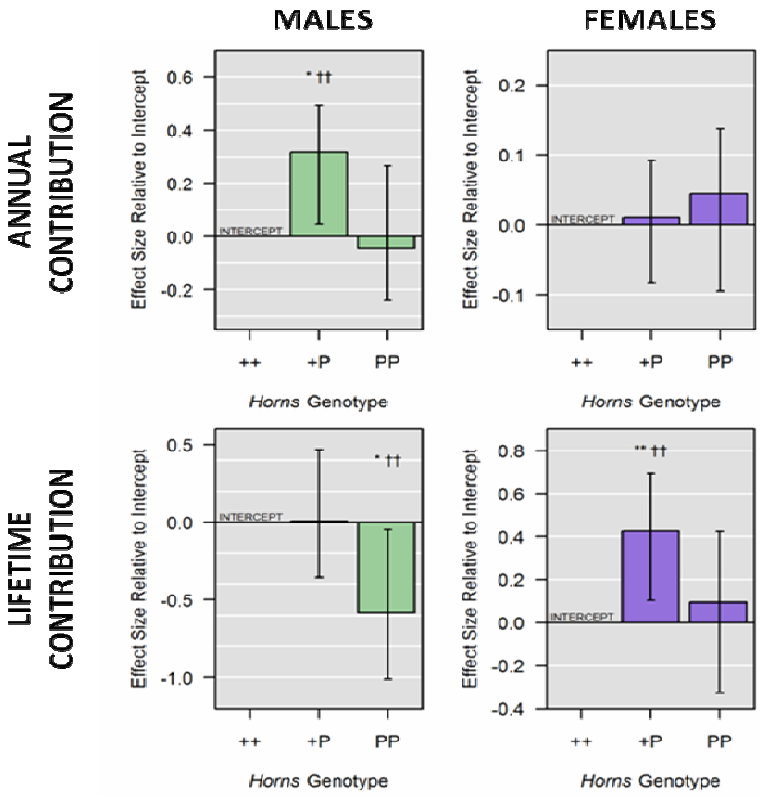

Figure 17. The annual and lifetime contribution to population growth in males and females for each Horns genotype. *The effect sizes are given relative to the contribution for the genotype* ***++.*** ** = 95%; ** = 99% confidence that the mean is significantly different from that of* ***++.*** *†† = 99% confidence that the mean is significantly different from that of* ***PP****.*

Individual variation in recombination rates revisited.
Jon Slate, Harriet Mellenius, Dario Beraldi & Josephine Pemberton.

In the 2007/8 Annual Report we described an analysis of recombination rate in Soay; recombination rate is the extent to which crossing-over between homologous chromosomes occurs during meiosis. Obtaining reliable estimates of recombination rate variation in a natural population is very hard as it requires detailed molecular data to be collected over several generations of related animals. However, data of this kind would be extremely valuable because, despite an enormous body of theoretical work about the evolution of recombination rate (which is a closely related to the evolution of sex), there are very few empirical datasets with which to test theory.

In the earlier report we described how recombination rate in Soay sheep was variable, how this variation was heritable and how it appeared to be under selection. Those analyses suggested that selection favoured individuals with *low* recombination rate, which was surprising because recombination rate appeared to be *increasing* during the course of the long term study. However, we warned that those analyses were preliminary.

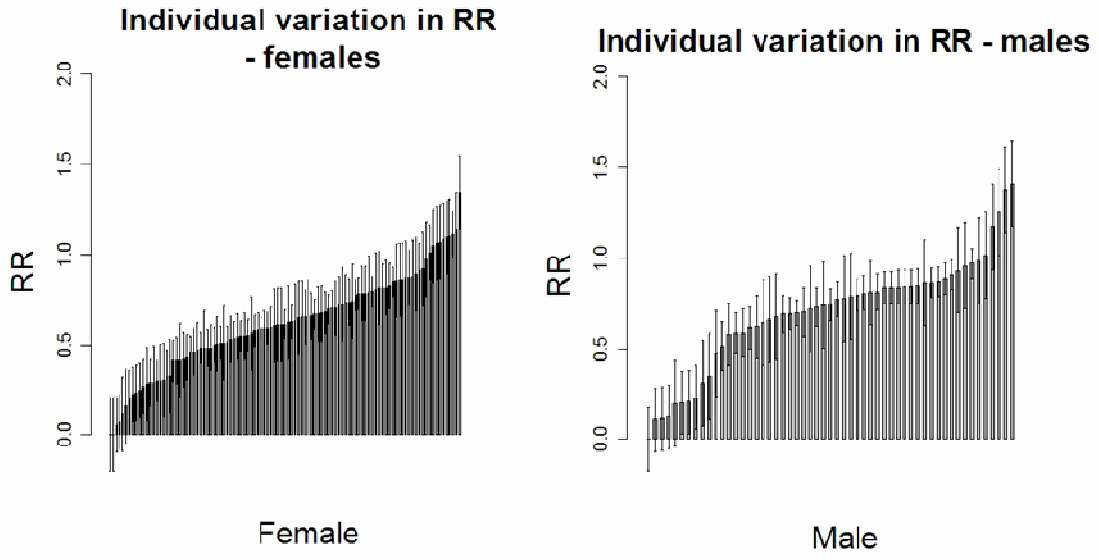

Figure 18. *Individual Variation in Recombination Rate (RR). Note that individuals vary substantially in how much they recombine.*

We have now examined the data in more depth in order to establish whether there were any biases in the way we were measuring recombination rate. In particular we were concerned that the number of genotyped ancestors an individual had might influence our power to detect how much recombination was occurring in its gametes. Our new analysis did indeed reveal some biases, but nonetheless some interesting results were upheld. We now know that recombination rate is variable (Fig. 18) and that this variation is heritable (estimated proportion of variation due to heritable effects ~ 0.41, 95% credible interval = 0.25-0.58). We can also confirm the temporal trend for increasing recombination rates (Fig. 19). The original finding that recombination rate variation was associated with low fitness was not upheld however. Instead, there is marginally significant trend for fitter individuals to have more recombined gametes (coefficient = 0.009; 95% CI = -0.001 – 0.018; P = 0.076). Therefore, the counterintuitive evolutionary trend that we described in 2007/8 is no longer present. If selection for

increased recombination rates is causing an evolutionary response then this effect is mainly driven through males, as males have higher recombination rates and the association between fitness and recombination rate is stronger in males.

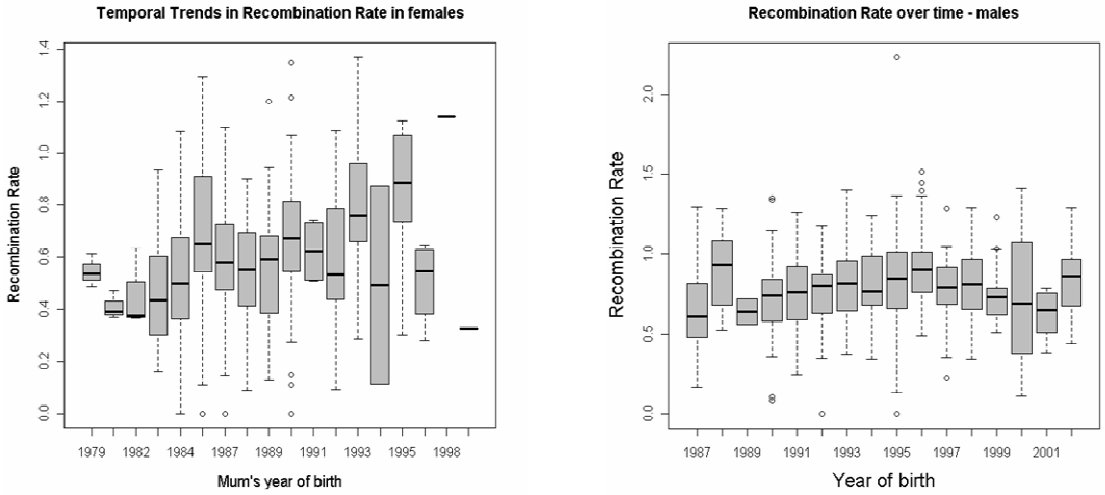

Figure 19. *Temporal Trends in Individual Recombination Rate. In both sexes there is evidence that recombination rate is increasing. Females P = 0.01, males, P = 0.08,*

The dataset we used for this analysis (~250 microsatellite markers typed in ~500 indivduals) is one of the best of its kind in any natural population. However, the forthcoming Soay sheep SNP chip genotyping effort will enable us to produce a considerably bigger and better analyses, and we are hopeful that the biases that we are aware of will be further reduced in the larger dataset. In the meantime, we are confident that our data will provide excellent empirical data to test theories regarding what has been described as the *'The queen of problems in evolutionary biology'* (Bell, 1982).

St Kilda Mouse Project: Year 1
Tom Black.

The St Kilda field mouse project aims to produce a cohesive overview of the ecology and evolutionary history of *Apodemus sylvaticus hirtensis*, a poorly studied race of mice endemic to the archipelago. Although much work remains to be done, some preliminary data is available from the five intensive live-trapping sessions undertaken across 12 months in three locations (Carn Mor, Glen Bay and Village Bay) on the main island of Hirta.

Population dynamics:
The St Kilda field mouse populations studied have fluctuated in size over the last year in a manner typical of small mammals in seasonal climates (Fig. 20). Population sizes reach their peak in late autumn as the last weaned juveniles from the summer breeding window (Fig. 21) enter the active population. There is considerable winter and spring mortality, with some sites showing up to a ten-fold difference in mouse density between autumn and early summer trapping sessions. There are often significant differences in mouse density between sites, but these are inconsistent and the underlying factors driving them are not yet known.

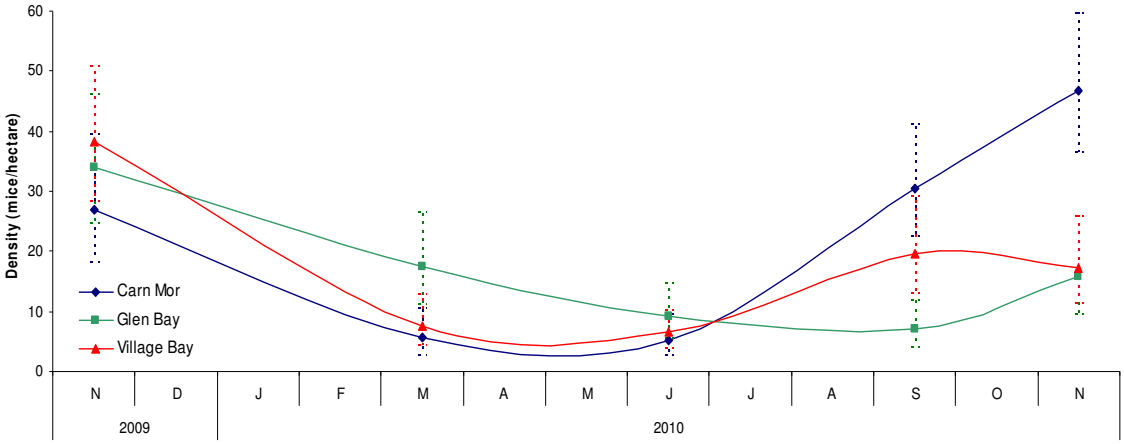

Figure 20. *Number of mice per hectare for three sites over a 12 month period, calculated using inverse prediction from spatially explicit capture-recapture data, with 95% confidence intervals.*

Supplementary trapping on the nearby island of Dun has confirmed the presence of mice there, where they appear to be have a higher density than on Hirta, presumably due to the increased availability of food items such as seeds due to the lack of sheep grazing.

Breeding ecology:

The 2010 breeding season was at its peak between June and September (Fig. 21). Although evidence of recent mating was occasionally seen throughout the year, juvenile mice only entered the population during the summer, suggesting that matings at other times of year do not result in offspring being born or reared. The 2010 breeding season appears to be have been slightly shorter than reported in a previous study from 1969, but it is common for field mouse reproduction to vary in response to environmental influences, particularly food availability.

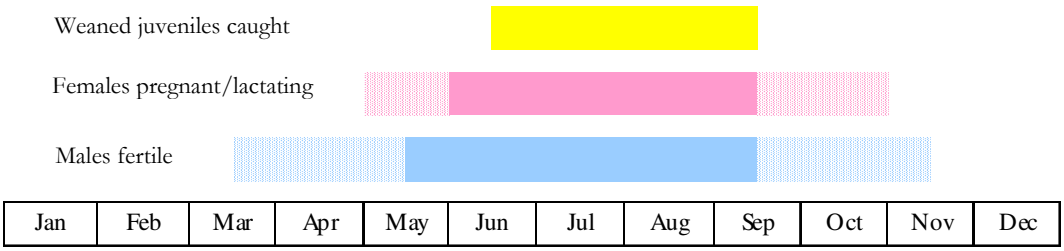

Figure 21. *Breeding season for 2010, based on observed and inferred reproductive status of individual mice.*

Weight distributions:

GLM analysis shows weight to vary significantly between grid ($p<0.000$, $F=14.42$, d.f.=2) sex ($p<0.000$, $F=47.38$, d.f.=1) and trapping session ($p<0.000$, $F=24.41$, d.f.=4). There was also a significant interaction between session and grid ($p=0.006$), $F=2.75$, d.f.=8), apparently as a result of juveniles entering the population at different times between grids. Figure 22 shows the distribution of weights of mice across the three grids for each trapping session. Where individuals were weighed on more than one occasion within a primary session, weights on first capture were used instead of means due to the

tendency for mice to lose weight over consecutive trap nights. Median weights are consistently higher in males than females across all grids and primary sessions, and there seems to be an overall pattern of mice being heavier on Carn Mor than Glen Bay or Village Bay. Despite considerable overwinter mortality, mice which survive normally put on weight between November and March and into the summer. The drops in weight distributions in September and November are as a result of juvenile recruitment.

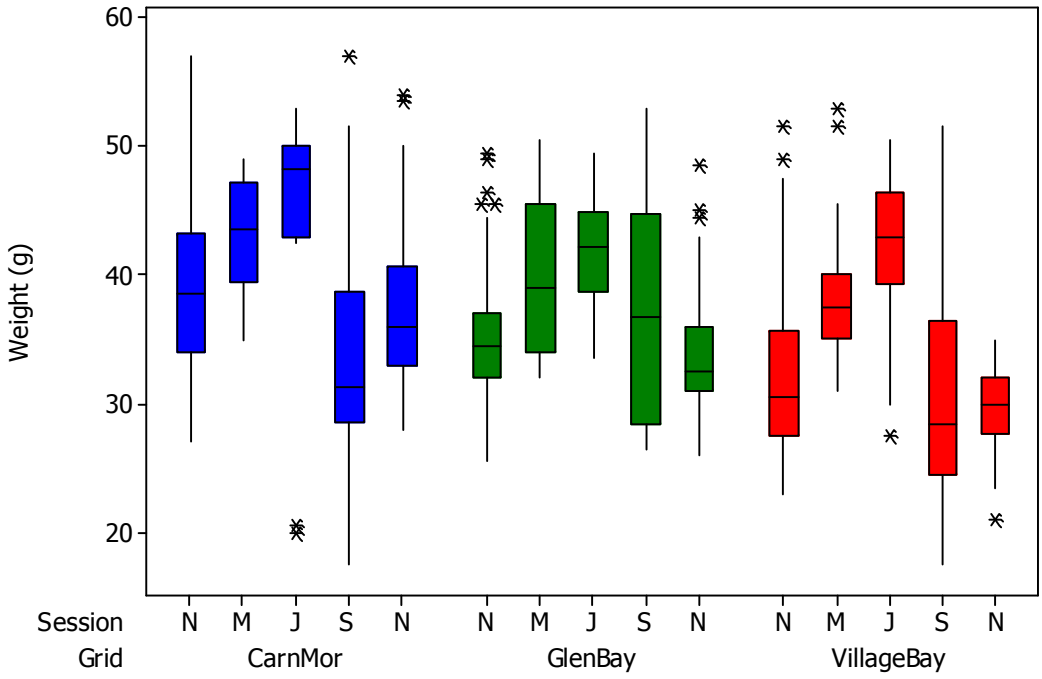

Figure 22. *Box and whisker plot of mouse weights pooled for all ages and sexes, by location and trapping session. Mid-lines indicate medians, boxes upper and lower quartiles, whiskers indicate smallest and largest values except for outliers (*) greater than 1.5x inter-quartile range.*

The trapping sessions have also yielded a large number of blood and tissue samples, which will be used for genetic analysis of individual breeding success, localised genetic differentiation and evolutionary history and for individual level stable isotope analysis of diet. A morphometric study using museum and present day specimens is also underway to determine if the mice have changed size over the last century.

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In press:

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ACKNOWLEDGEMENTS

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We are also grateful for the help of volunteers without whom the fieldwork for 2010 would not have been possible: Desiree Allen, Gebre Asefa, Katy Bell, Sophie Bell, Marcus Brent-Smith, Ronan Dugan, Callum Gilhooley (Lamb Catching Centurion), Becky Hewitt, Annelies Leeuw, Kathleen Morriss, Katy Orford, Gina Prior, Katie Stopher, Jamie Swannell, Lisa Thunstrom, Craig Walling and Andrew Wiles. Thank you.

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APPENDIX A: PERSONNEL NEWS & SCHEDULE OF WORK

Personnel News

Jill Pilkington has completed 20 years of field work on St Kilda and would like to thanks Frances Gulland for getting her involved in the first place, Andrew MacColl for leaning on the ferry rail at Castlebay and giving her the thought that she would like to, could and would do his job, many hundreds of volunteers, Project members and friends on St Kilda over the years, who have helped, advised and supported her through the good times and the bad, Bruce her husband for stoically supporting her, sending her a hand written letter (and a bar of chocolate) EVERY week, without fail, of those 20 years, Josephine Pemberton, to whom she set her cap at, back in the early 1990s, and doesn't regret it for a moment, and finally, of course the sheep, for whom her affection, though some times tested, has never failed.

Schedule of work on St Kilda

Winter - Spring

Jill Pilkington monitored mortality during 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 23rd until May 7th, Jill Pilkington, and 3 volunteers carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 241 lambs were born to 225 ewes; these figures include 16 sets of twins (15 ewes held both lambs, 1 lost one lamb). 174 lambs (78 male and 96 female) were caught and tagged; 7 lambs escaped capture; a further 67 lambs died before any tagging attempt. Mick Crawley and two assistants collected vegetation data.

Tom Black and one volunteer conducted trapping sessions in March and May on Hirta. He was able to land on Dun in May and also trapped mice there.

Summer

Jill Pilkington and two volunteers returned to Hirta on July 16th to carry out ten population censuses, conduct mortality searches (yielding 4 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. 368 sheep were caught and processed, of which 120 were lambs (48 males and 72 females), 65 were yearlings (27 males and 38 females), 41 were adult males, and 142 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 41 Sheep were retagged because of damaged or missing tags. 21 previously untagged lambs, 10 yearlings and 3 adults were caught and processed. Mick Crawley and two assistants collected vegetation data. Jill Pilkington and two volunteers remained on Hirta until 3rd September to complete parasite counts and pasture larvae counts.

Tom Black and one volunteer conducted trapping sessions in August/September.

Autumn

From October 19th to December 10th Jill Pilkington and two volunteers carried out ten population censuses, monitored the mating period, capturing and processing 31 incoming tups and 17 resident tups and ewes. 40 previously darted, non-resident tups were seen in the study area during this rut. 6 dead sheep were found.

Tom Black and one volunteer conducted trapping sessions in November/December.

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