ST. KILDA SOAY SHEEP & MOUSE PROJECTS:
ANNUAL REPORT 2009

J.G. Pilkington¹, S.D. Albon², A. Bento⁴, D. Beraldi¹, T. Black¹, E. Brown⁶, D. Childs⁶, T.H. Clutton-Brock³, T. Coulson⁴, M.J. Crawley⁴, T. Ezard⁴, P. Feulner⁶, A. Graham¹⁰, J. Gratten⁶, A. Hayward¹, S. Johnston⁶, P. Korsten¹, L. Kruuk¹, A.F. McRae⁹, B. Morgan⁷, M. Morrissey¹, S. Morrissey¹, F. Pelletier⁴, J.M. Pemberton¹, M.R. Robinson⁶, J. Slate⁶, I.R. Stevenson⁸, P. M. Visscher⁹, K. Watt¹⁰, A. Wilson¹, K. Wilson⁵.

¹Institute of Evolutionary Biology, University of Edinburgh.
²Macaulay Institute, Aberdeen.
³Department of Zoology, University of Cambridge.
⁴Department of Biological Sciences, Imperial College.
⁵Department of Biological Sciences, Lancaster University.
⁶Department of Animal and Plant Sciences, University of Sheffield.
⁷Institute of Maths and Statistics, University of Kent at Canterbury.
⁸Sunadal Data Solutions, Edinburgh.
⁹Queensland Institute of Medical Research, Australia.
¹⁰Institute of Immunity and Infection research, University of Edinburgh.
The sheep population on Hirta entered 2009 at a high level and, as a result, there was a slightly higher level of mortality than normal in a non-crash year. 100 tagged sheep were found dead within the study area between March and May of 2009. Lambing began on the 20th of March with 78% of lambs born surviving (Fig. 1).

Figure 1. The temporal distribution of lamb births during 2009.

In December 2009, 748 tagged sheep were believed to be alive on Hirta, of which 617 regularly used the study area, a total increase of 11% 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 2009.
Figure 3. The number of tagged sheep regularly using the study area since 1985.

One whole-island count yielded 2208 tagged and untagged sheep, with the details displayed in Table 1. The total population had increased by 15.6% since summer 2008, when it was at 1909. This gives a delta (calculated as \( \ln \left( \frac{N_{t+1}}{N_t} \right) \)) of 0.146.

Table 1. Demographic and geographic distribution of sheep observed during the count of Hirta on August 17th 2009. Coat colours are DW = dark wild, DS = dark self, LW = light wild, and LS = light self.

<table>
<thead>
<tr>
<th>Location</th>
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<th>Males</th>
<th></th>
<th>Lambs</th>
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<tr>
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</table>
Vegetation.
Mick Crawley.

The season of 2008-09 will be remembered as the crash-that-never-happened. Very high sheep numbers in August 2008 combined with extremely low food availability in summer 2008 led us to think that the population was heading into the winter of 2008-09 in dire condition. Not only did the population fail to crash but, following a successful lambing in spring 2009, numbers increased to produce the highest total island count we have ever seen (there were 2208 animals in August 2009). The population appears to have been rescued from its anticipated fate by unusually high grass productivity through the autumn and winter of 2008, followed by above-average grass growth in spring and summer 2009. On the bright side, these results emphasize how important plant productivity can be as an explanatory variable for sheep population dynamics.

A really important, but still unanswered question, is why the whole island sheep population is steadily increasing in size, year on year (Fig. 4). For the current study (post 1985) the average increase in population is more than 36 extra animals per year (p < 0.001). For the initial study (1954-68) the annual increase was roughly the same, at more than 34 animals per year, but for this shorter time-series, the trend falls short of significance (p = 0.095). Combining the data from the two studies produces a highly significant (p < 0.00025), but intriguingly shallower trend (with just under 13 extra animals per year; the dotted line in Fig. 4). We need to know what is driving this trend. It is plausible that plant productivity is increasing over time with climate change, and/or that the sheep are eating more of the vegetation biomass, or converting the plant biomass they eat into animal numbers more efficiently.

Figure 4. Time series in sheep numbers (solid symbols) during the first study period (1954-68) and the current study period (1985-2009), showing the trends in population size within the two periods (solid regression lines) and averaged across the two study periods (dashed line; see text for details). No matter how we measure the trend, it is clear that the whole island population is increasing as the years go by.
One way of getting a handle on this is to look for matching long-term trends in the vegetation data. It is plausible that the upward trend in sheep numbers is associated with long-term declines in one or more components of the vegetation. It turns out that there has been a highly significant decline in August mean tussock mass in the inbye grasslands since 1994 (when this data series starts; \( p = 0.0002 \); Fig. 5). There has been no significant trend in mean gap mass over the same period (negative slope, but \( p = 0.29 \)). It is no surprise that the biomass of tussocks is more responsive to fluctuations in sheep numbers than is the biomass of the perennially closely-cropped, lawn-like gaps. The trend in individual tussock mass correlates closely with a significant (\( p = 0.00047 \)) long term negative trend in total August biomass (where tussock and gap mass are weighted by the proportional cover of gaps and tussocks). Similar patterns were observed in March biomass, but with a rather less significant decline in tussock total mass (\( p = 0.043 \)) and, again, no decline in gap mass (\( p = 0.08 \)).

**Figure 5.** Average total dry plant mass (per 0.04m\(^2\)) in August for the grasslands inside the Head Dyke (inbye) in tussocks (upper solid line) and gaps (lower dotted line). There is a significant decline over time in the biomass of tussocks (\( p < 0.001 \)) but no significant trend in the biomass of gaps (\( p = 0.29 \)). Note that both tussocks (solid line) and gaps (dotted line) were relatively high in August 2009 (the right-hand ends of the two time-series point upwards) despite record high sheep numbers, reflecting the high grass productivity despite high offtake in the preceding months.

As you can see (Fig. 5) the August 2009 biomass data from both gaps and tussocks buck the long-term downward trend, and these positive residuals occurred in the face of the highest island sheep count ever made (2200 animals). Coupled with this, the March 2009 tussock and gap biomasses, measured at the height of the crash-that-never-happened, were somewhat below their long-term trends, lending support to the hypothesis that it was enhanced vegetation productivity rather than increased biomass that rescued the population from crashing.
Weather during population crashes.
Ana Bento and Mick Crawley.

We need to know why it is that winter NAO (the North Atlantic Oscillation) features so often in our models of sheep population dynamics on St Kilda. We know that NAO is correlated with “kinds of winters”, but why is it that a pressure differential between Iceland and the Azores explains patterns of population dynamics on a Hebridean Island? Surely we could do better by using the weather measured locally on St Kilda to interpret the observed patterns of birth and death? To this end, we correlated winter NAO with all the locally measured weather variables from the three St Kilda stations (installed at the end of 1999), and discovered that the only significant correlation of NAO was with February rainfall ($r^2 = 0.61$, positive relationship; $p < 0.008$): two other terms, marginally significant on their own (atmospheric pressure in February (negative; $p = 0.02$) and total run of wind in February (positive; $p = 0.03$)), did not survive model-simplification when February rain was included in the model.

This made a lot of sense. We have used February rainfall in models in the past, and it is intuitively appealing that dismal weather at the time of year when the animals are at their most vulnerable would be correlated with elevated death rates.

The next thing we did was to calculate average February rainfall in the original study period (1952-1969; Met. Office data) and in the current study (1985-2009; Met office data to 1999 then our AWS data). Intriguingly, the mean February rainfall in the current study period is much higher than it was during the 1950s and 60s (124 mm vs 79 mm; $p = 0.002$). Our working definition for a crash for Soay sheep on St Kilda is a decline in the (base e) logarithm of total sheep count from one August to the next of -0.7 or more. There have been four of these so far: one in the initial study period (1959-60) and three in the current study period (1988-89, 1998-99 and 2001-02). We worked out mean rainfall in February for the crash and non-crash years separately, and found that February rainfall was much higher in the crash year than the mean of the 13 non-crash years for the original study period. We then looked at the three recent crashes, and the pattern was the same; there was significantly higher average mean February rainfall in the three crash years than in the 20 non-crash years. A simple picture was emerging: high-density populations apparently crash in wet Februaries, but not in average or dry Februaries (Fig. 6).

However, if we broaden the definition of a crash to include declines of -0.6, then two more winters enter the picture, 1966-67 and 1985-86. The decline in 1966-67 during the initial study period conforms to the wet February hypothesis. But February 1986 was the driest ever, with only 16mm of rain measured for the entire month. Other Hebridean weather stations confirm this result, so it wasn’t something anomalous about the St Kilda station that year.
Figure 6. Average total rainfall during February (mm) in years with different sheep population dynamics. The light bars show February rain in years when the population crashed, and the dark bars show the average rainfall in February of non-crash years. The two left-most bars relate to the initial study (1954-1968), the next two bars to the current study period (since 1987), and the right-most bar shows the exceptionally dry February of 1986. Bars show plus and minus 1 standard error of the mean. Note how much higher February rainfall has become between the first and second study periods in both crash and non-crash years.

So there you have it. Most, but not all, population declines occur in wet Februaries. Armed with this new insight, we can re-visit recent Februaries when the population was high but did not crash (e.g. 2007 and 2008). It turns out that these two years would clearly have been classed as extremely wet Februaries during the early period of the study (with 152 and 158 mm respectively; see Fig. 6), and therefore should have crashed if high February rain really was the proximate cause of these extreme population declines. Whereas we know that high population density, an elderly-biased age structure and low grass productivity predict a crash, there does not seem to be single, simple, weather variable that makes the difference between a crash or a non-crash.

Sexual conflict in Soay twins.
Peter Korsten, Tim Clutton-Brock, Jill Pilkington, Josephine Pemberton and Loeske Kruuk.

Males and females are generally very different in morphology, physiology and behaviour, enabling each sex to successfully survive and reproduce. In Soay sheep, for example, males grow to larger body size, have bigger horns, and show more aggressive behaviour compared to females. As a result, the two sexes may also have different requirements during their early development, even before birth. In mixed-sex twins this could potentially lead to a negative influence between individuals of different sex while still in the womb. Males are already larger than females at birth, which suggests that male embryos may be more efficient in the competition for maternal resources than females. Lab studies, mainly on rodents, have also shown that male embryos produce and require higher levels of male sex hormones, such as testosterone, during their development than females. These male hormones may leak through the membranes enclosing the embryos and may harm the development of neighbouring females. In the Soay sheep, we studied the effects of co-twin sex on both weight at birth and the number of lambs produced by female twins over their lives. Relatively few studies have investigated short- and long-term effects of co-twin sex simultaneously, particularly not in wild animals. Free-living animals like the Soay sheep are
more likely to be resource limited than animals kept in captivity, which may amplify the potential conflict over resources between co-twins of opposite sex.

Previous research on the Soay sheep has shown that twinning rates vary considerably between years and the likelihood of twinning also depends on the age and condition of the mother. Approximately 15% of births are twins, while the other 85% are singletons. Virtually all twins are dizygotic (i.e. come from separate eggs). We restricted our analysis of co-twin sex effects to the weights of lambs captured and measured within one week of birth (of these, 89.0% were captured within three days) and we controlled for the variation in capture age and several other variables known to influence lamb weight, like birth date and the mother’s age and weight in the preceding August. We found a 10.0% (0.18 kg) reduction in birth weight of female lambs with a male co-twin relative to those with a female co-twin, while the weight of males only differed by 1.4% (0.03 kg) depending on the sex of their co-twin (Fig. 7; lamb sex × co-twin sex interaction: \( p = 0.006 \)). Previous work on the Soay sheep has already shown that having a twin sibling substantially reduces birth weight (see also Fig. 7). Now we show that there is additionally a sex-specific effect of having a twin sibling: female birth weight is more reduced by the presence of a male co-twin, than by a female co-twin, while for males the reduction is not related to their co-twin’s sex.

**Figure 7.** Mean (± SE) birth weight of Soay sheep lambs in relation to the composition of the litter in which they were born. ‘F’ indicates female, ‘M’ male. The statistical analysis only included twins (354 lambs in 184 litters born to 119 mothers). Values for singletons are depicted for visual comparison only.

Furthermore, the presence of a male co-twin appears to have long-term fitness consequences for female twins, because females with a male co-twin produced significantly fewer lambs over their lifetimes (lifetime breeding success) than females with a female co-twin (Fig. 8; \( p = 0.013 \)). This reduction in lifetime breeding success remained significant after taking into account birth weight, which had a significant positive effect on lifetime breeding success (\( p = 0.004 \)). The difference in lifetime breeding success between females with male versus female co-twins seems to be partly driven by differences in first-year survival. When added to the model, first-year survival explained significant variation in lifetime breeding success (\( p < 0.001 \)), and after taking account of this effect, the effect of co-twin sex
was no longer significant ($p = 0.090$). It is possible that sex-specific competition over maternal resources (e.g. milk) after birth also contribute to the observed differences in lifetime breeding success of females in relation to their co-twin’s sex. This seems quite likely, given that the effect on lifetime breeding success was partly driven by differences in first-year survival.

These results suggest there are appreciable short- and long-term effects of co-twin sex on a female sheep. Next, we will investigate the likely mechanisms causing the effect: resource competition and/or hormonal interference, and we will investigate the effects of co-twin sex on male lifetime breeding success.

Maternal effects and early-life performance are associated with parasite resistance across life in free-living Soay sheep.
Adam Hayward, Jill Pilkington, Josephine Pemberton, and Loeske Kruuk.

The inheritance of characteristics through genetic inheritance is perhaps the central tenet of biology. However, in addition to direct effects of genes, the decisions and behaviour of the mother can have an important influence on offspring. Such influences are known as ‘maternal effects’, and there is much evidence to suggest they have an impact, not only in the offspring’s immediate chances of surviving, but that they may also influence offspring long after offspring have left maternal care. It has also been shown that decisions made early in life may be associated with an individual’s own performance in later life.
Maternal effects can have a profound influence on the ability of offspring to resist parasites. For instance, mothers may provide protective antibodies through the placenta or the first milk (colostrum) in mammals. Soay sheep on St Kilda are infected by a group of intestinal worms known as strongyle nematodes, which cause reduced body condition and mortality; the number of strongyle parasites infecting a sheep can be estimated by counting worm eggs in the faeces of sheep. We investigated effects of maternal effects and early life performance on parasite resistance across the lifespan of Soay sheep, using almost five thousand strongyle parasite counts collected over 24 years. We aimed to identify maternal effects associated with parasite resistance in lambs, and to assess the extent to which these associations persisted into adulthood. We also looked for influences of maternal effects on the rate of deterioration in parasite resistance with increasing age.

Our results suggested that twins had higher parasite counts at four months of age than did lambs born as singletons (Fig. 9). Also, adult males born as twins not only had higher strongyle parasite counts than those born as singletons, but those born as twins also experienced higher levels of parasite infection as they got older, whereas adult males born as singletons experienced only a small increase as they aged (Fig. 10).

Surprisingly, we found that adult females born as twins had lower parasite levels than adult females born as singletons (not shown). A possible reason for the difference between males and females is their different life history - males are usually susceptible to parasites because they put as much effort into reproduction as possible, whereas females show higher resistance to parasites. Females born as singletons generally achieve higher reproductive success than females born as twins, who may therefore invest more in parasite resistance than reproduction and so be more resistant than adult females born as singletons.

A further finding was that lambs of young and old mothers had more parasites than lambs of middle-aged mothers. This effect was most pronounced in male lambs, in particular suggesting that male lambs with older mothers are less resistant to parasites (Fig. 11). This suggests that older mothers are less able...
to provide parasite resistance, possibly through protective antibodies in colostrum, since this effect is present even after accounting for the fact that older mothers may give birth to lighter and weaker lambs. The higher parasite levels of lambs born as twins and of adult males born as twins is also consistent with this hypothesis.

We also found that early-life performance in females is associated with changes in parasite resistance with age in late life. Older females which experienced large parasite infections earlier in life had increasingly high parasite levels as they aged (Fig. 12a). Finally, our results suggested that females which reproduce at high levels in early life showed declining parasite burdens as they aged, whereas females which were less successful in early life showed increasing parasite levels as they aged (Fig. 12b). This is suggestive of a positive association between early and late life performance. Interestingly, we also found that females which began reproducing earlier in their lives showed higher parasite counts as adults, which is suggestive of a trade-off. These results therefore highlight the complexity of the relationship between reproduction and immunity.
Figure 12a and b. a) Senescent females which experience high FEC in their first summer are predicted to experience a more rapid increase in FEC in late life than those experiencing low FEC in early life; b) Females with high fecundity in early life are predicted to show decreasing FEC in later life, while those with low fecundity show increasing FEC.

This study is the most comprehensive analysis of associations between maternal phenotype and early life performance and parasite resistance in a free-living vertebrate. We have shown that maternal effects are an important influence on lamb parasite resistance, but also that maternal effects may persist into adulthood. We have also shown that early life parasite resistance and reproductive performance are associated with varying rates of ageing in the ability to resist parasites, and illustrated the complex relationship between parasite resistance and reproduction.

Autoimmunity in Soay sheep on St Kilda.
Andrea Graham, Adam Hayward, Kathryn Watt, Jill Pilkington, Josephine Pemberton and Dan Nussey.

Autoimmunity occurs when the immune system attacks the body's own cells and their constituents instead of parasites. Autoimmune diseases, such as arthritis and multiple sclerosis, can result from this kind of malfunction in the immune system. These diseases are difficult to treat and are on the rise in many countries. Recent studies of both humans and laboratory rodents have identified immune genes that seem to predispose the individual to high levels of autoimmunity and a high risk of autoimmune disease. From an evolutionary point of view, this is a puzzle. We might expect that, since autoimmune disease has seriously detrimental consequences for health, natural selection would have eliminated genetic variants that were predisposed to such disease. A possible explanation may be that autoimmunity is actually an unfortunate side-effect of a very active and responsive immune system. In natural, parasite-filled environments, having a highly responsive immune system may bring benefits in the form of improved protection against disease, which may balance the costs of raised immunity. To date, no study has investigated autoimmunity in the wild and therefore we know little about how natural selection actually acts on it.

We adapted tests for a very general marker of autoimmunity (anti-nuclear antibodies or “ANA”), which is widely used in clinical settings, for use with Soay sheep blood samples. We measured ANA in over
2,500 samples collected from Soay sheep in the Village Bay population on Hirta, St Kilda during the August catches in 1997-2007. The sheep showed levels of ANA comparable to those observed in clinical studies of humans. In human medicine, ANA levels above a certain threshold are sometimes used to identify patients at risk of autoimmune disease in future: a form of ‘early warning’ of a potential health problem. Around 25% of around 400 adult female Soay sheep tested scored above this threshold (Figure 13). We were also able to show that female Soays varied markedly in their ANA levels and that a significant part of this variation was genetic (around 25%). As expected, we also found evidence that autoimmunity was correlated with other measures of immune responsiveness. Interestingly, we did not find that high ANA was associated with reduced survival as might have been expected if it were associated with autoimmune disease. Instead, females with high average ANA were longer lived, as might be expected if ANA were reflecting wider immune activity and protection against parasites and disease.

![Figure 13](image)

**Figure 13.** Scatter plot of adult female and male ANA optical densities relative to plate-specific background controls (with points jittered for each sex; note only the maximum ANA score per individual is plotted). Unbroken lines are the median values for each sex and the broken line is the threshold for positivity for the ANA assay

Although high ANA was associated with improved survival in female Soay sheep, it was negatively associated with other traits. Females with high ANA were more likely to have failed to breed the preceding spring and, if they did breed, were more likely to have produced small lambs. These antagonistic associations between autoimmunity and survival and reproduction may help explain how and why genetic variation in both autoimmunity and also wider immune function persists in mammals.
A pilot study to find genes contributing to variation in Soay sheep body size
Josephine Pemberton, Dario Beraldi, Peter Visscher, Hong Lee, Allan McRae, Jake Gratten and Jon Slate.

In the last three years there have been dramatic strides in our knowledge of the domestic sheep genome, from which our studies of Soay sheep are going to benefit enormously. Specifically, a collaboration of researchers, principally in Australia, New Zealand and the USA, and known as the International Sheep Genomics Consortium (ISGC) have sequenced DNA from multiple sheep breeds in order to find variable sites in the DNA, known as single nucleotide polymorphisms. This work culminated in the production of a chip (an array of DNA segments the size of a microscope slide) capable of testing the genetic make-up of a sheep at 60,000 known polymorphic SNPs scattered evenly throughout the sheep genome.

In the summer of 2008 we were invited to participate in a programme to screen a wide variety of sheep breeds with this chip. This programme has multiple objectives, including the identification of primitive segments of DNA and the study of breed relationships (see the following report for an example). But one of the chief purposes of such a chip is to locate genes underlying variation in traits of interest.

In Soays, we are especially interested in body size, because we consistently find selection for larger body size but consistently observe that the sheep are getting smaller. Hind leg length is one of the most heritable traits we study, with 40% of the variation in adults due to genetic variation. If we could find genes underlying body size variation in Soays, we would be able to investigate directly how the population does or does not respond to selection on body size at the genetic level. When submitting DNA samples to the ISGC for genotyping, we therefore selected individuals which were at the extremes of the distribution of leg length within our database. In the end we obtained data for 486 Soays divided equally between the extremes of the distribution.

Of the 60,000 SNPs on the chip, no less than 38,000 of them are polymorphic in Soay sheep, an unexpectedly high proportion given the remoteness and small size of the population! This places the Soay sheep in a unique position in relation to other studies of wild animals – we can assay genetic variation with far greater intensity in our population than any other equivalent project. Second, we conducted a genome-wide association analysis looking for SNPs that might be linked to one or more genes associated with leg length. For this we have screened only chromosomes 1-26 and not the X or Y (yet). Results are shown in Fig 14. At one end of chromosome 16, four consecutive SNP loci show very high likelihood of being linked to such a gene. In cattle, the equivalent region of the genome contains a gene called Iroquois homeobox protein 1 (IRX2) and in mice mutations in this gene are associated with variation in limb growth. It is a very strong candidate gene for explaining variation in leg length in Soay sheep. We will be investigating this gene and others nearby to try and find the exact basis of the leg length variation so that we can genotype many Soays for it and follow selection and its effects at the genetic level.

This study shows that genome-wide association analysis holds promise for the detection of genes underlying genetically-varying characteristics in wild animal populations.
Soay sheep and admixture.
Philine Feulner, Jake Gratten and Jon Slate.

In the annual report for 2005 we raised the idea that Soay sheep may have experienced some mixing (termed admixture by geneticists) with more modern breeds. The evidence came partly from written records, with some tentative population genetic support. The advent of the sheep 60K SNP chip (see previous report) has allowed us to test this more rigorously.

In the 19th and early 20th centuries, Soay sheep were only found on Soay and the St Kildans farmed more modern breeds on Hirta. However, Donald Ferguson, the postmaster in the later 19th century, described how:

“A few rams of the race which preceded the introduction of the Blackfaced rams were once introduced into Soay but they did no good” (Elwes (1912) The Scottish Naturalist pp 25-29).

By the time the St Kildans left Hirta in 1930 they were farming Scottish Blackface sheep, but prior to that they farmed the Old Scottish Shortwool (sometimes called Dunface) breed, and it is this breed to which Ferguson refers. In other words, Dunface genes may have had the opportunity to mix with Soay sheep genes on Soay, prior to the introduction of just over 100 Soay sheep from Soay to Hirta in 1932. However, testing for admixture with genetic markers is difficult because the Dunface breed is extinct. Fortunately, the Boreray breed is known to be descended from both Dunface sheep and Scottish Blackface sheep, and so Dunface genes persist in contemporary Boreray populations.

In the 2008 report it was shown that Soays were the most distinct sheep in an analysis of 23 breeds typed at ~1,500 SNP genetic markers and there was no evidence for admixture. However, Boreray sheep were not included in that analysis. Both Soays and Boreray sheep have now been typed at almost 60,000 SNPs. To determine whether this larger dataset was capable of detecting admixture, if it has occurred, we first used simulations, in which Soays and Dunface underwent an admixture event approximately 30
generations (~120 years) ago. Both the real and simulated data were analysed using a population genetics software called STRUCTURE. This program takes genetic data, and then attempts to identify how many discrete genetic clusters or populations are present in the data. It is also capable of showing whether individual animals contain genes from one or more populations. We used data from four breeds – Soays, Borerays, Scottish Blackface and an African breed called Red Maasai, which were included as an outgroup.

The simulations showed that 1,500 markers would be insufficient to detect admixture, but 11,000 would be enough. Similar simulations, with no admixture, generated different patterns. In other words, the dataset is now sufficiently large to test for admixture (Fig. 14). When the real data were analysed, the results with 1,500 SNPs and 11,000 SNPs were almost identical to those generated by the simulated admixture models (Fig. 15). In other words, it appears that Soays have undergone admixture with another breed, most likely Dunface sheep in the late 19th century.

**Figure 14.** STRUCTURE analysis of simulated data. Each shade represents a different genetic cluster. Note that when no admixture is simulated, Soays form one discrete cluster. When admixture with Dunface is simulated, 1,500 SNPs are insufficient to detect it in Soays. However, when 11,000 SNPs are used, nearly all Soays are a mixture of original Soay genes and Dunface genes.
Figure 15. The real data show that 11,000 SNPs detect admixture in Soays but 1,500 SNPs do not, as predicted by the simulations of admixture.

If admixture has occurred, then an obvious question is whether it is responsible for much of the genetic variation occurring in the population today. We are currently investigating this question, and it seems possible that both the light coat colour and the self coat pattern appeared in the population as a result of admixture. If so, the dark wild phenotype can be regarded as the ancestral Soay type appearance. It is important to note that admixture should not be regarded as a process that has diluted the genetic integrity or value of Soay sheep. First, it remains the case that Soay sheep are genetically very different from all other breeds. Second, the introduced genes probably came from a now extinct breed, and so Soays are a ‘living museum’ of genetic variants that were common ~150 years ago. Finally, admixture is a very common, but hugely under-studied process in the natural environment. By comparing ancestral Soay and more modern Dunface genetic variants, we should be able to examine how selection acts on both wild-type and domesticated genes in the wild.

Mapping the Horns gene in Soay sheep.
Susan Johnston and Jon Slate.

Horns are a secondary sexual character present in all wild species of sheep. They play an important role in competition between males for access to females during the rutting season. Soay sheep are unusual in that they have inherited differences in horn type in both males and females. The majority of males have large, normal horns (88% of all records), whereas a small proportion of males (12%) have much smaller deformed, or ‘scurred’ horns. Female Soay sheep have smaller horns than males, and exhibit three phenotypes: normal horns (33%), scurred horns (35%) and a completely hornless, or ‘polled’ phenotype (31%). Previous research by Matt Robinson and other members of the project has shown that males with normal horns are more likely to win access to females during the rut, but have reduced longevity compared to scurred males. In females, those with scurs conceive more offspring, have greater weaning rates and better over winter survival than normal or polled females.
DNA evidence to date suggests that the presence of horns in domestic and Soay sheep is controlled by a single gene, *Horns*, which, although uncharacterised, has been mapped to chromosome 10 in domestic sheep, and to an overlapping region of chromosome 10 in Soay sheep, spanning approximately 7.4cM (where 1cM ≈ 1 million bases of DNA). Also, there is evidence for a gene contributing to variation in horn length in normal-horned males, which has been mapped in Soay sheep to a region overlapping the *Horns* locus. All this evidence suggests that having different copies of the *Horns* gene (i.e., a different genotype) may not only be responsible for variation in horn type, but also variation in horn size in normal-horned males. Until now, we have been unable to identify the *Horns* genotype in individual sheep. The following report describes our work to identify the *Horns* gene and determine whether it really does have an effect on horn length.

Colleagues at AgResearch Ltd. in New Zealand have used information from other species, such as humans, mice and cattle, to identify a candidate gene for *Horns*. In the process, they identified several ‘single nucleotide polymorphisms’ (SNPs) within and around the candidate gene in domestic sheep; SNPs are mutations in the DNA where a single base can differ between individuals in the population. We examined 21 of these SNPs in Soay sheep, and identified SNPs which were strongly associated with horn type, as well as SNPs which had a large effect on horn length.

We found that one SNP was strongly associated with horn type in both males and females. This SNP allowed us to determine how horn type is inherited, and which genotypes underlie each phenotype. It appears that there are two versions of the *Horns* gene in Soay sheep, named Ho*+* and Ho*P*. In males, *Horns* is likely to be a dominant gene, where carrying one or two copies of Ho*+* (i.e., an individual with the genotypes Ho*/Ho*/ or Ho*/Ho*P*) will result in normal horns. However, in animals with the genotype Ho*/Ho*P*, around half will develop normal horns and half will develop scurs. It is not yet clear what determines the different horn types within these males. In females, *Horns* is likely to be an additive gene, where Ho*/Ho*/ sheep have normal horns, Ho*/Ho*P* sheep have scurs and Ho*P*/Ho*P* sheep are polled.

We also looked to see if the genotype at *Horns* had an effect on horn length in normal-horned males. We did this by calculating how much of the variation in horn length was inherited (=28.4%), which in turn allowed us to calculate an estimated breeding value (EBV) of horn length for each normal-horned male. By comparing the median EBVs, we found that normal-horned males with the genotype Ho*/Ho* tend to have the longest horns, and normal horned males with the genotype Ho*/Ho*P* tend to have the shortest horns (Fig. 16A: ‘Polygenic’). We then calculated how much variation in horn length is due to the genotype at *Horns* (= 39.7%) and recalculated the EBVs. We found that when we take the genotype at *Horns* into account, there is no difference between the median horn length values in normal horned males with different genotypes (Fig. 16B: ‘SNP10’). This means that the *Horns* gene not only determines horn type in all sheep, but also contributes to variation in horn length in normal horned males.
Now that we have a genetic marker that distinguishes between sheep with identical phenotypes but different underlying genotypes, we are in a position to examine whether sheep with different Horns genotypes differ in their lifetime fitness, which in turn should enable us to understand how the different horn types are maintained in the population.

**Figure 16.** Boxplot of estimated breeding values (EBVs) for the predicted genotypes at the Horns gene for the ‘polygenic’ and ‘SNP10’ calculations. The thick black horizontal lines indicate the mean EBV for each predicted genotype.

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

Parasites present a constant challenge to individuals and populations, and are one of the main factors influencing survival and reproduction. However, despite the obvious benefit of fending off disease, immune responses vary widely and many hosts remain susceptible. Much work has focused on trying to understand how and why this variation occurs.

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 0.1 to 0.5. 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 to also 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 animal’s DNA is washed over the chip and the bits of interest bind to it, whilst the remainder is washed away. 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 I used a variety of resources, using 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 122 candidate genes, and also 50 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. 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). The DNA was sent to the University of Liverpool Advanced Genomics Facility, where the sequence capture and subsequent sequencing was carried out.

Five hundred million base pairs of sequence data was returned in December, and I can now compare variation in Soay sheep immune genes between the two groups, and also examine whether any difference in variation between groups is greater at immune genes rather that at the random genes. Any interesting SNPs can later be typed in many individuals, and associations between these SNPs and resistance, and later fitness, can be examined.

**Analysis 2: Other sheep breeds.**
The NimbleGen experiment should provide an indication of what genes are involved in resistance to gastrointestinal nematodes in Soay sheep. It is also possible to address the question of whether there is a common set of genes that explain variation in resistance within and between breeds, using data made available by the sheep SNP chip project.
To do this, it is first necessary to gain a measure of resistance for different sheep breeds. 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 whether lambs or ewes are used, or whether sheep are left to graze naturally or are artificially infected with parasites. Using a statistical model which accounts for these different factors, a standardised measure of FEC for the different breeds can be obtained. Sufficient information to carry out such an analysis was available for 17 breeds: 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. These breeds are all included in the International Sheep Genomics Consortium project, and so data for 49,000 SNPs are available for these breeds.

To identify SNPs that are under selection, a measure of genetic differentiation called $F_{ST}$ is used. SNPs under selection are expected to have atypical levels of genetic differentiation (they are sometimes called ‘outlier loci’). Loci under directional selection, for example, should show a greater $F_{ST}$ than neutral loci, whereas loci under balancing selection should show a lower $F_{ST}$. Among the outlier loci, those which best explain inter-breed variation in FEC will be identified. If these SNPs are found in the candidate genes identified in Analysis 1, then this may suggest that there is a common set of genes explaining resistance to gastrointestinal nematodes. However, if they are found elsewhere in the genome then Soay sheep and other breeds are likely to have a different genetic basis to resistance.

By the next annual report, we should have made considerable progress in answering this question.

**Ecology and evolution of the St Kilda field mouse.**
Tom Black.

The only mammal other than sheep to found on the archipelago, the St Kilda field mouse (*Apodemus sylvaticus*) has previously been given both species and subspecies status on the basis of the morphological differences between it and mainland field mice. Approximately twice as heavy as mainland mice and with different fur colouration, surprisingly little is known about the St Kilda field mice, despite them being endemic to a World Heritage Site. Previous trapping efforts in the 1900s, 1930s and 1950s yielded some basic morphological and distributional data, but there have been few quantitative records pertaining to the mice for over fifty years.

Over the next three years this project will investigate several aspects of the ecology and evolution of the St Kilda field mouse, with the intention of informing future management plans for the islands (in terms of both the mice and the species with which they interact) and increasing understanding of the mouse’s evolutionary history. In particular, the following areas will be investigated:

*Population distribution, size and dynamics*
Knowing these is key to being able to properly consider the mice in the archipelago’s management plans, as well as providing insight into the environmental variables that may limit their populations (there is apparently little predation pressure or inter-specific niche competition on the islands). This will be assessed via repeated live-trapping sessions on three sites across Hirta, as well as opportunistic trapping and chew block surveys at other locations around the archipelago.
Breeding ecology
Repeated trapping sessions, individual identification and genotyping will be used to determine breeding season and construct pedigrees which will allow measurement of individual breeding success and estimations of dispersal rates which can feed into population models.

Diet
Stable isotope analysis will be used to monitor switching between prey sources and construct mixing models of relative prey intake. In particular, suspected use of sheep carcasses and seabird eggs (especially the declining Leach’s storm-petrel) as food sources will be examined.

Genetic affiliations between St Kilda field mice and other A. sylvaticus
Although almost certainly introduced to St Kilda by man several thousand years ago, the origin of the field mice is not known for certain. Genetic comparisons with field mice from the Scottish mainland, islands and Scandinavia will be used to attempt to trace the history of colonisation. This work will build on and collaborate with that of J. Herman at the National Museums of Scotland.

Are St Kilda field mice getting larger over time?
Morphological comparisons (using dental wear to control for age) will be made between modern St Kilda mice and museum specimens dating back over 100 years to determine if the mice are still undergoing changes in size.

Activity to date
The first field season in November 2009 established three long-term trapping grids on Hirta (located in Village Bay, Carn Mor and Glen Bay). Each 0.81 hectare square grid consists of 100 trapping stations at 10m intervals, with 2 live traps per station (1 per station at Carn Mor this season). Trapping sessions will take place four times a year, for five consecutive nights. Caught mice will be individually marked, measured and sampled, then released where they are caught. The dietary, genetic and long-term morphological study components will begin in 2010.

During this first field season a total of 154 individually identified mice (70 males, 84 females) were caught across all grids over 13 trap nights. Recapture rates were high with 480 combined capture events. Most mice showed distinct home ranges, moving an average of 12-17 metres between trapping occasions and showing a strong preference for areas containing walls, cleits and complex scree and rock formations. The majority of mice caught appeared to have been born in the same year, with very few fully-grown individuals, suggestive of high yearly mortality. No juveniles or visually pregnant females were caught, although a few females did show signs of having recently mated. There were significant weight differences between sexes and trapping grids (Fig. 17), the former being attributable to differences in body length as males were longer than females on average (uncorrected for age). Weight differences between grids may be due to temporal differences in sampling or mouse breeding strategies; further sampling should allow estimation of mouse age for correction.
Figure 17. Box-and-whisker plots of body weight of male (M) and female (F) field mice at Carn Mor (CM), Glen Bay (GB) and Village Bay (VB) grids. Figure shows median body weights (lines), 25% to 75% quartiles (boxes) and ranges (whiskers). Outliers (*) are shown for extreme values greater than 1.5 times the inter-quartile range of the box. For mice caught more than once, mean body weight measurements were used.

Publications


In press:


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APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK

Personnel Changes

Andrea Graham and Kathryn Watt conducted extensive assays of immune system molecules in stored plasma samples and analysed the data in relation to life history data (see ‘Autoimmunity in Soay sheep’ above). Sheena Morrissey genotyped the 2009 lambs and other previously untyped sheep at 18 microsatellite loci for paternity analysis. Tom Black started a PhD on ‘The Ecology and evolution of the St Kilda field mouse’.

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 10th until May 8th, Jill Pilkington, Peter Korsten, Emily Brown and a volunteer carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 222 lambs were born to 208 ewes; these figures include 14 sets of twins (12 ewes held both lambs, 2 lost one lamb). 169 lambs (78 male and 91 female) were caught and tagged; 5 lambs escaped capture; a further 48 lambs died before any tagging attempt. Mick Crawley and two assistants collected vegetation data.

Summer

Jill Pilkington and two volunteers returned to Hirta on July 14th to carry out ten population censuses, conduct mortality searches (yielding 1 tagged dead animal), and prepare for the main catch-up of study area sheep. The catch-up took place from August 6th – 22nd, was led by Josephine Pemberton, and conducted by a team of 11 additional project members and volunteers. 303 sheep were caught and processed, of which 111 were lambs (57 males and 54 females), 45 were yearlings (15 males and 30 females), 33 were adult males, and 114 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 14 Sheep were retagged because of damaged or missing tags. 15 previously untagged lambs, 1 yearling and 1 adult were caught and processed. Mick Crawley and two assistants collected vegetation data. Jill Pilkington and two volunteers remained on Hirta until 4th September to complete parasite counts and pasture larvae counts.

Autumn

From October 17th to December 5th Jill Pilkington and two volunteers carried out ten population censuses, monitored the mating period, capturing and processing 18 incoming tups and 23 resident tups. 40 previously darted, non-resident tups were seen in the study area during this rut. 2 dead sheep were found.
CIRCULATION LIST  -  (Please advise J.Pilkington of any changes or additions)

Prof. S. Albon  Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Ms. S. Bain  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr. D. Bancroft  GPC AG, Lochhmer Str. 29D-82152, Munich, Germany.
Mr. A. Bennett  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Ms. A. Bento  Dept. Biological Sciences, Imperial College. Silwood Park, Ascot, SL5 7PY.
Dr. D. Beraldi  Roslin Institute Edinburgh Univ., Roslin, Edinburgh EH25 9PS.
Ms. E. Brown  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
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  Prof. Of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
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, Y041 1LZ.
Prof. M. Crawley  Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. S. Davies  SNH, Fraser Darling House, 9 Culduthel Road, IV2 4AG.
Dr. T. Ezard  Dept. Biological Sciences, 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. P. Feulner  Westfälische Wilhelms Univ., Inst. Evol. and Biodiv., Hüfferstrasse, 148149 Münster, Germany.
Dr. J. Gratten  Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
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.
Ms. S. Johnston  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. O. Jones  Inst. Zoology, ZSL, Regent’s Park, London NW1 4RY.
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. D. MacLennan  SNH, 17 Frances St., Stornoway, Lewis, Outer Hebrides.
Ms. C. Mazzetta  Dept. Statistics, University of Warwick, Coventry, CV4 7AL.
Mr. A. McRae  Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. J. Milner  Hogskenol i Hedmark, Evenstad, NO2480, Koppeg, Norway.
Prof. B. Morgan  Inst. Maths.& Stats., Univ. Kent., Canterbury, Kent, CT2 7NF.
Mr. S. Murray  Craigie Dhu, Cardney, Dunkeld, Perthshire, PH8 0EY.
Dr. A. Ozgul  Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. S. Paterson  School of Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. F. Pelletier  Dept. Biologie, Univ. of Sherbrooke, Quebec, Canada, J1K 2R1.
Dr. B. Preston  Max Planck Inst. Evol. Anthropology, 04103 Leipzig, Germany.
Dr. M. Rees  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. M. Robinson  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
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. Tavecchia  Imedea-CSIC/UIB, c. M. Marques 21, 07190 – Esporles, Mallorca, Spain.
Dr. L. Tempest  7 Mandrake Road, London, SW17 7PZ.
Dr. P. Visscher  Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. S. Votier  Sch. of Biomed. & Biol. Sci., Davy Building, Drake Circus, Plymouth, Devon, PL4 8AA.
Ms. K. Watt  Inst. Immunity & Infection, Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Dr. K. Wilson  Dept. of Biological Sciences, Lancaster University, LA1 4YQ.