ST. KILDA SOAY SHEEP PROJECT:
ANNUAL REPORT 2004


1Institute of Evolutionary Biology, University of Edinburgh.
2Macaulay Institute, Aberdeen.
3Department of Zoology, University of Cambridge.
4Department of Biological Sciences, Imperial College, University of London.
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.
8Institute of Biological Sciences, University of Stirling.
9Sunadal Data Solutions, Edinburgh.
10Biology Department, Penn State University, U.S.A.

POPULATION OVERVIEW .................................................................................................................. 2
REPORTS ON COMPONENT STUDIES................................................................................................ 4
The genetic basis of coat colour variation in Soay sheep: a candidate gene approach .............................. 4
Mapping quantitative traits in Soay sheep ................................................................................................ 5
How precise are estimates of relatedness bases on genetic markers? ...................................................... 6
Birth weight, birth date and litter size: whose phenotype is it and does it matter? ................................. 9
Faecal hormone metabolite dynamics of Soay rams on St. Kilda .......................................................... 12
Gastro-intestinal nematode intensity in crash 2002 sheep .................................................................. 15
Vegetation ........................................................................................................................................... 18
PUBLICATIONS................................................................................................................................. 20
ACKNOWLEDGEMENTS .................................................................................................................... 21
APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK ...................................................... 21
CIRCULATION LIST ............................................................................................................................ 23
The sheep population on Hirta entered 2004 at a moderately high level and, as a result, in the first few months of the year there was a slightly higher level of mortality than normal in a non-crash year. Thirty two sheep were found dead within the study area between January and May of 2004. Lambing recruitment began on the 26th of March with 73% of lambs born surviving (Fig.1).

**Figure 1. The temporal distribution of lamb births during 2004.**

In December 2004, 812 tagged sheep were believed to be alive on Hirta, of which 671 regularly used the study area, a total increase of nearly 36% on the previous year. The age distribution of the population is shown in Fig. 2 and changes in sheep numbers in the study area over time are shown in Fig. 3.

**Figure 2. Age distribution of tagged Soay sheep presumed to be alive at the end of 2004.**
Figure 3. The number of tagged sheep regularly using the study area since 1985.

One whole-island count yielded 1996 tagged and untagged sheep on the whole island with the details displayed in Table 1. The total population had increased by 21% since summer 2003, when it was at 1568.

Table 1. Demographic and geographic distribution of sheep observed during the count of Hirta on August 14\textsuperscript{th} 2004. Coat colours are DW = dark wild, DS = dark self, LW = light wild, and LS = light self.

<table>
<thead>
<tr>
<th>Location</th>
<th>Females</th>
<th>Males</th>
<th>Lambs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW</td>
<td>DS</td>
<td>LW</td>
<td>LS</td>
</tr>
<tr>
<td>Conachair/Oiseval</td>
<td>166</td>
<td>9</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>Mullach Bi/Cambir</td>
<td>230</td>
<td>11</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>Ruaival/Village</td>
<td>293</td>
<td>16</td>
<td>121</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>689</strong></td>
<td><strong>36</strong></td>
<td><strong>259</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>
REPORTS ON COMPONENT STUDIES


Jake Gratten, Bethan Lowder and Jon Slate.

Coat colour is polymorphic in Soay sheep on St Kilda. There are four recognised colour morphs: dark wild, light wild, dark self and light self. ‘Wild’ refers to the wild-type, or mouflon pattern, with pale coloured rump, stomach and facial markings. ‘Self’ means that coat colour is uniform all over the body. Doney (1974) proposed a model for coat colour variation in Soay sheep involving two biallelic autosomal loci, Brown at which dark (B+) is dominant to light (Bb) and Agouti at which wild (A+) is dominant to self (Aa). In a study of 556 lambs with known maternity and paternity inferred at 95% confidence, Coltman & Pemberton (2004) showed that this two locus model is a good fit to the observed inheritance of dark/light and wild/self in Soay sheep. Here we report on a candidate gene approach to discovering the genetic basis of coat colour variation in Soays. We aim to (i) identify the genes responsible for dark/light colouration and wild/self patterning in Soays, (ii) determine if these genes are under selection, and (iii) investigate how selection maintains genetic variation.

We selected four candidate genes for dark/light colouration from the melanin synthesis pathway. The best characterised of these is melanocortin 1 receptor (MC1R), which is known to be responsible for colour variation in birds (e.g. chicken, bananaquit) and many mammals, including humans, mice, pigs, horses, cattle, and domestic sheep. The other dark/light candidates included: tyrosinase related protein 1 (TYRP1), which is associated with colour variation in cattle & dogs; dopachrome tautomerase (DCT), which plays an essential role in the pathway leading to eumelanin (dark pigment); and microphthalmia-associated transcription factor (MITF), which is known to activate TYRP1 and DCT, causing a switch from light to dark pigment production. For wild/self patterning we considered only one gene, Agouti because wild-type Soays have characteristic ‘agouti’ bands on their hairs, whereas self-type animals do not.

Our approach involved sequencing a single intron (stretch of DNA) per candidate gene in order to identify one or more single nucleotide polymorphisms (SNPs). We then typed these SNPs in ~5 individuals of each colour morph and looked for an association with coat colour or pattern, assuming tight linkage between the intronic SNPs and the causal mutations in the coding or regulatory regions of the genes. This method enabled us to rapidly screen multiple candidate genes, as opposed to sequencing the entire coding region of each gene. Our analysis revealed that TYRP1 is associated with dark/light colouration and Agouti is associated with wild/self colour pattern in Soay sheep (Table 2). We will confirm the results of the association study by demonstrating linkage in the mapping pedigree between TYRP1 and dark/light, and Agouti and wild/self. Assays for intronic SNPs are in hand, although if linkage disequilibrium is incomplete with these markers we may repeat the analysis using the causal SNPs.
Table 2. Tests for association between candidate genes and coat colour traits in Soay sheep.

<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>Coat colour trait</th>
<th>No. sheep</th>
<th>( X^2_{(df)} )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>MC1R</em></td>
<td>Dark/light</td>
<td>21</td>
<td>1.04(_{(2)})</td>
<td>0.5958</td>
</tr>
<tr>
<td><em>TYRP1</em></td>
<td>Dark/light</td>
<td>20</td>
<td>22.00(_{(4)})</td>
<td>0.0005</td>
</tr>
<tr>
<td><em>DCT</em></td>
<td>Dark/light</td>
<td>20</td>
<td>1.78(_{(4)})</td>
<td>0.7692</td>
</tr>
<tr>
<td><em>MITF</em></td>
<td>Dark/light</td>
<td>20</td>
<td>3.73(_{(2)})</td>
<td>0.0796</td>
</tr>
<tr>
<td><em>Agouti</em></td>
<td>Wild/self</td>
<td>15</td>
<td>8.19(_{(2)})</td>
<td>0.0167</td>
</tr>
</tbody>
</table>

We are in the process of sequencing the coding regions of *TYRP1* and *Agouti* to determine the molecular genetic basis of coat colour variation. *TYRP1* has eight exons (seven translated) spanning some 18Kb, and *Agouti* has four (3 translated), so to simplify the sequencing process we are working from complementary DNA (cDNA), rather than genomic DNA. We collected ear punches during the 2004 summer catch, isolated total RNA and then synthesized cDNA by reverse transcription. Sequence analyses will be combined with a quantitative PCR-based analysis of candidate gene expression in wild-type and self-type sheep, to investigate the possibility that regulatory mutations are responsible for coat colour phenotype. Finally we will conduct molecular evolution analyses to determine if coat colour genes are under selection.

Mapping quantitative traits in Soay sheep.

Dario Beraldi, Peter Visscher, Jon Slate, Allan McRae, Josephine Pemberton.

Most of the characteristics of an organism result from complex interaction between many genetic loci and the environment. These characteristics can be referred to as quantitative traits (QTs) and can be analysed by a combined statistic and molecular approach. Provided that QT measurements and pedigree information are known, the impact of the genetic component on a QT can be determined by means of statistical tools, such as mixed models, that remove from the data (i.e. from the QT) the non genetic variation. The genetic component can be then dissected to investigate if and how some genomic regions affect a QT more than others. This analysis is called quantitative trait mapping. This task is accomplished by following the inheritance of alleles at a large number of molecular markers through the pedigree and then testing for a statistical association between one or more markers and the QT. A molecular marker is a DNA sequence for which the position in the genome is known and which is polymorphic with a number of recognizable variants (alleles) among different individuals.

In this project we aim to map the genomic regions that affect different morphological traits (birth and body weight, hind leg length, coat colour, horn type), resistance to gastrointestinal nematodes and fitness in Soay sheep. The long term goal is to provide a framework to better understand how evolution takes place and how genetic variation is maintained in the face of natural selection.

At present, about 50% (1672 cM, 10 chromosomes) of the Soay sheep genome has been mapped with 117 markers screened on a pedigree of 588 individuals.
The genetic locus determining the coat colour (dark or light in Soay) was found on chromosome 2 (closest flanking marker at 2.3 and 4.3 cM) in a region where a strong candidate gene (TYRP1) is known to be located (see Fig. 4). This result is strong support for the results reported in the previous report (Gratten et al) that TYRP1 is responsible for the dark/light polymorphism.

**Figure 4.**

**a)** Soay chromosome 2 map showing in detail the region harbouring the coat colour locus. This region is syntenic to cattle chromosome 8 where the TYRP1 gene (associated with coat colour variation in cattle) has been mapped.

**b)** Likelihood ratio profiles for chromosomes 2 and 5 showing peaks for parasite resistance. Markers used for the scan are represented below the X axis.

A preliminary scan for parasite resistance, a trait measured as count of nematode eggs in a faecal sample, suggests a region on chromosome 2 and on chromosome 5 responsible for this trait, the chromosome 2 region corresponding to the location of the coat colour gene. Although these results are preliminary, it will be interesting to formulate and test a hypothesis to explain the maintenance of genetic variation at the parasite resistance regions and the possible correlation with coat colour.

**How precise are estimates of relatedness based on genetic markers?**

Katalin Csilléry and Josephine Pemberton.

Knowledge of genetic relatedness between pairs of individuals in natural populations is enormously useful for answering all kinds of questions, for example: is cooperative behaviour associated with high relatedness and driven by kin selection? Do individuals avoid mating with relatives? Are populations structured according to relatedness? To what extent is continuous variation in a trait inherited (i.e. what is its heritability)? Observing mother-offspring relationships, and inferring father-offspring relationships using genetic markers in order to build up pedigrees, as we do in the Soay sheep and red
deer studies, is an approach which is not available to many other studies for reasons of time, expense or practicality. Instead, many projects use genetic markers such as DNA microsatellites to estimate the relatedness between pairs of individuals in the population, using one of a number of different relatedness estimators which have been published over the years. All methods calculate how many alleles two individuals share over a sample of markers, and then use different techniques to refine the estimate using information about the overall level of variation at the locus, of the frequency of the different alleles.

Marker-based estimates of relatedness have proved very successful in some contexts. They are at their best when mean relatedness for groups can be tested against each other, for example in meerkat groups, mating between a dominant female and subordinate males is strongly associated with relatedness: non-breeding subordinate males are on average related to the female, while breeders are not, providing evidence of incest avoidance in this species (Griffin et al., 2003, Behavioral Ecology 14:472-480).

However, the statistical properties of relatedness estimators are not very satisfactory and there is continuous development of improved estimators. In particular, the precision with which the marker-based estimators estimate true relatedness between pairs of individuals is low, potentially making marker based estimates poor when used as explanatory variables. One issue which has not been extensively addressed is how well-correlated pedigree and marker-based relatednesses are in free-living populations. We are investigating this issue in a series of long-term, individual-based studies including the Rum red deer and St. Kilda Soay sheep populations. We predict that the correlation will depend on the amount of genetic information available (number and polymorphism of marker loci), but may also vary with the relatedness structure of the populations. Specifically, in populations where the mean and variance in pedigree relatedness between randomly drawn pairs of individuals is low (generally because most pairs of individuals are unrelated), we would expect the correlations between marker-based relatedness and pedigree relatedness to be poor.

In order to standardise the accuracy of pedigree relatedness, we selected individual red deer and Soay sheep from the datasets which had the following characteristics: all four grandparents had been identified and there were no mismatches between the focal individual and its parents at any of the marker loci. This selection procedure resulted in 72 focal red deer and 92 focal Soay sheep. The pedigree for each individual was used to trace and calculate its pedigree relatedness to other individuals in the sample. Figure 5a and b show the distribution of relatedness values found in the sample and reveals that a much higher proportion of the deer are related to each other than is the case in the Soay sheep population.

We then estimated relatedness between focal individuals with six different marker-based relatedness estimators, using 8 marker loci of high variability in the deer and 15 loci of medium-low variability in the Soay sheep. Figure 5c and d show the correlation between pedigree relatedness and one of the most commonly-used markers-based estimators, invented by Queller and Goodnight. Rather shockingly, only 14% (deer) and 10% (Soays) of the variance of pedigree relatedness is detected by the marker-based estimator. The greater variability of the deer marker loci and the higher variance in pedigree relatedness seem likely to explain the difference between the species. This is preliminary work: there are many further investigations to be made on this issue, some of which may
improve the explanatory power of the marker-based estimators, but many of which seem likely to make it worse.

In conclusion, our study shows that in populations such as ours where the mean and variance of pedigree relatedness is generally low (as compared with, say, a honeybee colony), marker based relatedness may be only weakly correlated with pedigree relatedness, and therefore a poor proxy for pedigree-based relatedness in analyses of cooperation, inbreeding avoidance, population structure and quantitative genetics.

**Figure 5.** Frequency distributions of pedigree relatedness in focal samples of (a) red deer on Rum and (b) Soay sheep on St. Kilda, indicating that in the deer population there are many more related pairs of individuals in the population. Correlations between marker-based relatedness (Queller and Goodnight estimator) and pedigree-based relatedness for (c) red deer and (d) Soay sheep, indicating the poor precision with which markers estimate relatedness. Focal individuals for the study were selected to standardise the amount of pedigree-based information according to criteria described in the text.
Birth weight, birth date and litter size: whose phenotype is it and does it matter?

Alastair Wilson & Loeske Kruuk.

A trait is expected to evolve if it has a heritable component of phenotypic variance and is also under selection. In the Soay sheep of Hirta, previous analyses of lamb weight and size traits have found significant heritabilities and evidence of positive directional selection. Nevertheless, there is little evidence for increasing lamb size over the study period. However, offspring size is also an important component of female reproductive effort and can therefore be treated as part of the maternal (rather than offspring) phenotype. We hypothesized that the evolution of offspring size might be constrained by antagonistic effects (either genetic or selective) acting at offspring and maternal levels.

For example, it is known that heritable maternal effects can have important consequences for trait evolution, and in particular that a negative genetic correlation between maternal and additive genetic effects will act to dampen any response to selection. There is evidence that this effect is important in domestic livestock, but maternal genetic effects have never been tested for or quantified in evolutionary studies. Additionally, while selection on lambs and mothers may be reinforcing, there is also potential for antagonism if offspring and maternal fitness are optimized at different lamb sizes. We therefore tested for maternal genetic effects on lamb size, and also determined the nature of selection acting through both offspring and maternal fitness. Birth weight was used a measure of lamb size, and since this trait is phenotypically correlated with birth date and litter size (singletons being born heavier and later) these traits were also considered simultaneously in a multivariate framework.

Quantitative genetic analyses were performed using the Animal Model approach. We fitted (and compared using likelihood ratio tests) a sequence of successively more complex models (Table 3) to estimate heritable sources of phenotypic variance including both additive and maternal genetic effects as well as the correlation between them \( r_{AM} \). Under the best model for each trait (Model 3 for birth weight and birth date, Model 4 for litter size), heritabilities \( h^2 \) were low while maternal genetic effects \( m^2 \) represented a large proportion of the total phenotypic variance for each trait (birth weight \( m^2 = 0.119 \), birth date \( m^2 = 0.197 \), natal litter size \( m^2 = 0.211 \)). Under Model 4, a negative correlation between direct and maternal genetic effects was estimated for each trait, but was only statistically significant for natal litter size \( r_{AM} = -0.714 \). Incorporating variance from heritable maternal effects and the direct-maternal genetic covariance into “Total Heritability”, \( h^2_T \) yielded estimates that were significant for birth weight and birth date but not for natal litter size (Table 4). Using multivariate animal models significant genetic correlations were estimated between birth weight and both birth date \( r_G = +0.962 \) and litter size \( r_G = -0.677 \).
Table 3. Animal models fitted for each trait.

<table>
<thead>
<tr>
<th>Model</th>
<th>Effects Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Additive</td>
</tr>
<tr>
<td>2</td>
<td>Additive + Maternal</td>
</tr>
<tr>
<td>3</td>
<td>Additive + Maternal Environment + Maternal Genetic</td>
</tr>
<tr>
<td></td>
<td>((\text{(r_{am})}) assumed zero)</td>
</tr>
<tr>
<td>4</td>
<td>Additive + MaternalEnvironment + Maternal Genetic</td>
</tr>
<tr>
<td></td>
<td>((\text{(r_{am})}) estimated)</td>
</tr>
</tbody>
</table>

Table 4. Estimates of total heritability \(h^2_T = (\sigma^2_a + 1.5\sigma_{am} + 0.5\sigma^2_m) / \sigma^2_p\) for Birth Weight, Birth Date, and Natal Litter Size from univariate Models 1 to 4 (standard errors are given in parentheses).

<table>
<thead>
<tr>
<th>Model</th>
<th>Trait</th>
<th>BIRTH WEIGHT</th>
<th>BIRTH DATE</th>
<th>NATAL LITTER SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODEL 1</td>
<td></td>
<td>0.349* (0.042)</td>
<td>0.355* (0.036)</td>
<td>0.382* (0.036)</td>
</tr>
<tr>
<td>MODEL 2</td>
<td></td>
<td>0.116* (0.046)</td>
<td>0.127* (0.040)</td>
<td>0.077* (0.037)</td>
</tr>
<tr>
<td>MODEL 3</td>
<td></td>
<td>0.135* (0.045)</td>
<td>0.197* (0.038)</td>
<td>0.102* (0.037)</td>
</tr>
<tr>
<td>MODEL 4</td>
<td></td>
<td>0.112* (0.047)</td>
<td>0.180* (0.042)</td>
<td>0.052* (0.039)</td>
</tr>
</tbody>
</table>

* Estimates based on the most appropriate model

* Significantly different from zero at \(p<0.05\) based on estimated standard errors.

Using several different measures of fitness, general linear modelling and regression analysis showed that all three traits were under selection (results not shown). Analysis of Lifetime Breeding Success \(W_{LBS}\); the number of offspring produced by an individual during its lifetime) revealed strong positive directional selection on birth weight, and weaker selection for increased birth date at both offspring and maternal levels, apparent from the positive gradients of the phenotype–fitness functions (Fig. 6). However, there was also evidence for stabilizing selection on these traits at the maternal level, with reduced fitness at high phenotypic values indicating lower phenotypic optima for mothers than for offspring (Fig. 6). Selection was antagonistic at offspring and maternal levels for litter size. From the offspring’s point of view it is better to be born a singleton, while mothers with higher average litter size do better over their lifetime. In fact the decreased fitness of twins is caused entirely by their reduced birth weight, and therefore this antagonistic selection results from a trade-off between litter size and birth weight that has different fitness optima for lambs and their mothers.
Figure 6. Univariate fitness ($W_{LBS}$) phenotype relationships for offspring and mothers. Solid lines depict cubic spline functions. Offspring fitness shows directional selection on birthweight and date, and maternal fitness shows directional selection on litter size. However, maternal fitness suggests stabilizing selection on birth weight and birth date.

Taken together, the results of these analyses provide considerable insight into important genetic and selective processes that will shape the evolutionary trajectories of these traits in the Soay sheep (Fig. 7). There is evidence for maternal genetic effects on all three traits, and the negative estimates of $r_{AM}$ suggest a possible mechanism by which evolutionary responses may be damped. Furthermore, a particularly striking result is the extent to which inadequate models (with no maternal effect fitted) overestimated trait heritabilities (by a factor of up to 6.45 in the case of birth date). Genetic correlations between the three traits will also limit the ability of each to evolve independently, and, most notably the negative $r_{G}$ between birth weight and litter size provides evidence of an important trade-off. The selection analysis shows that this trade-off has different optimal solutions to maximize fitness in lambs and mothers. For a lamb it is better to be born as a heavy singleton, while a mother does better by producing larger litters, even at some cost to the weight of her lambs. While we have therefore identified a number of important pathways and cross-generational effects (Fig. 7), a major task remaining is to integrate these findings into a quantitatively predictive model that can be empirically tested.
Figure 7. Genetic and selective pathways shaping evolution of birth weight, birth date and litter size. $V_A$ denotes additive genetic variance, $V_M$ denotes maternal genetic variance.

Faecal hormone metabolite dynamics of Soay rams on St. Kilda.

Brian Preston, Steve Monfort, Gerald Lincoln, Ian Stevenson and Ken Wilson.

Measurement of testosterone (T) metabolites in faeces is desirable as it allows us to monitor hormone profiles of rams without the use of invasive and labour-intensive techniques that may have undesirable effects on both the hormone being monitored and the behaviour subsequently exhibited. Previous attempts to incorporate faecal T assays into our study on St. Kilda have been frustrated by a lack of correspondence between plasma and faecal T temporal profiles (K. Wilson unpublished report); whilst plasma T measurements indicated that T levels in males were highest at the onset of the rut, declining to a minimum by its end (a pattern also observed in mainland studies by Gerald Lincoln), faecal T measurements suggested that T levels mirrored the availability of oestrous females, which peak mid-rut. A more detailed comparison of faecal and plasma T was hampered by the lack of ‘matched’ samples available from the same individual. However, limitations on the specificity of radio-immuno assays (RIAs) for T metabolites in faeces were identified as a likely source of error in faecal T assays.

With funding from a Royal Society Project Grant (KW), a team led by Brian Preston visited St Kilda in Rut 2003, specifically to collect matched faecal and plasma samples, and to collect repeat faecal samples from known individuals soon after deposition on the pasture. In February 2004, Brian Preston visited Steve Monfort’s lab. at the Smithsonian National Zoological Park, U.S.A., and, after extraction of faecal steroid metabolites and appropriate laboratory validation, he assayed faecal samples for T using an ELISA-based method. Plasma samples were assayed in Gerald Lincoln’s lab. at the MRC Edinburgh using the previously established methodologies (RIA). Three separate lines of evidence
indicate that assaying faecal T metabolites using ELISA provide a good estimate of circulating T levels:

(1) Temporal trends in T levels and behaviour.
Using the ELISA method for determining faecal T profiles, similar temporal trends are now apparent in both plasma and faecal T estimates. These now both indicate that T levels peak in rams close to the onset of the rut at the start of November, and progressively fall to a minimum at the end of the rut in early/mid-December. The temporal trends in T are closely associated with temporal trends in the percentage of time males spent in agonistic encounters with competitors, but are unrelated to the numbers of oestrous females in the study area or the time males spent engaged in sexual activity.

(2) Correlation between measures of plasma and faecal T using matched samples.
Matched plasma and faecal samples were collected from captured rams of all ages, at different times of each day and evening. These matched samples provide strong evidence that measurement of faecal T using ELISA provides a good estimation of current T levels, as assays of matched samples of plasma T and faecal T metabolites are highly correlated (Fig. 8). Because of the time-lag between the secretion of T into the bloodstream, and the excretion of its metabolites in faeces, disparate diurnal patterns in T secretion and metabolite excretion might influence the degree of association between the different T assays. However, we found no evidence that the correlation between faecal and plasma T is affected by the time of day that samples were collected across all levels of faecal T. We investigated whether ram phenotype or age influenced the association between plasma and faecal T. We failed to detect any influence of the age or body mass (condition) of rams on this relationship. Thus, comparison of faecal T assays appears to be justified for rams of all ages and condition in our sample.

(3) Effects of temporary chemical castration on faecal T.
In a previous study, led by Ian Stevenson, we temporarily excluded 10 males from the rut using a short-acting progestogen (MPA). This results in the temporary suppression of T in treated animals and potentially provides an additional route for validation of the faecal T assay methodology. Detection of decreased T levels in faeces of MPA treated males, relative to control rams, would provide additional evidence that the newly implemented ELISAs have good specificity for T metabolites. Behavioural observations on MPA-treated males showed that the rutting behaviour of these males had been almost entirely inhibited, indicating that the MPA treatment had been effective in suppressing T secretion. Analysis of faecal T assays concurred: T levels detected in faecal samples collected from MPA-treated males at corresponding periods throughout the rutting period were, on average, around half those of the control rams (Fig. 9). These results provide experimental validation of the faecal T assay methodology.

Overall conclusion.
Using faecal T metabolites to assay T secretion in the field offers great promise. The strong concordance between T estimates assayed using faeces and plasma samples from the same animal (Fig. 8); the strong relationship between faecal (and plasma) T and the amount of time engaged in agonistic behaviour; plus the effect of MPA on faecal T and male behaviour (Fig. 9) all appear to validate the faecal T method.
**Figure 8.** Correlation between estimates of T levels in the plasma and faeces for matched samples collected during rut 2003. The correlation between the two assay methods is strong, with the amount of variation explained exceeding 45% ($P<0.0001$).

**Figure 9.** Effects of MPA on male faecal T estimates. MPA injection results in a reduction in T metabolites in the faeces.
Gastro-intestinal nematode intensity in crash 2002 sheep.

Barbara Craig, Jill Pilkington and Josephine Pemberton.

In the population crash of 2002, full gastro-intestinal sampling of helminth parasites was performed on a subset of sheep for the first time since 1989 (Frances Gulland). Mean counts of each nematode species are given in Table 5. From the data it is clear that three species predominated, however, the relative abundance of *Teladorsagia* spp., *Trichostrongylus axei* and *Trichostrongylus vitrinus* varied differently with host age class than previously reported. Data were analysed using generalised linear models with negative binomial error structure and host sex and age as explanatory variables.

**Table 5.** Mean burden (± 95% C.I.) of all adult, gastrointestinal nematode species from different age classes of sheep that died in the crash of 2002.

<table>
<thead>
<tr>
<th>Part of intestine</th>
<th>Nematode species</th>
<th>Lambs (n=24)</th>
<th>Yearlings (n=13)</th>
<th>Two Year olds (n=19)</th>
<th>Adults (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abomasum</td>
<td><em>Teladorsagia</em> spp.</td>
<td>4,179±735</td>
<td>4,782±1442</td>
<td>9,863±2899</td>
<td>6,723±2985</td>
</tr>
<tr>
<td></td>
<td><em>Trichostrongylus axei</em></td>
<td>10,042±2171</td>
<td>9,709±4848</td>
<td>3,724±1315</td>
<td>1,285±793</td>
</tr>
<tr>
<td>Small intestine</td>
<td><em>Trichostrongylus vitrinus</em></td>
<td>8,917±2205</td>
<td>6,615±4682</td>
<td>4,361±1749</td>
<td>5,285±5841</td>
</tr>
<tr>
<td></td>
<td><em>Nematodirus battus</em></td>
<td>175±77</td>
<td>46±40</td>
<td>355±233</td>
<td>100±55</td>
</tr>
<tr>
<td></td>
<td><em>Nematodirus filicollis</em></td>
<td>1,571±778</td>
<td>615±427</td>
<td>89±127</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Bunostomum trigonocephalum</em></td>
<td>17±16</td>
<td>31±45</td>
<td>79±50</td>
<td>138±115</td>
</tr>
<tr>
<td></td>
<td><em>Strongyloides papillosus</em></td>
<td>13±19</td>
<td>0</td>
<td>16±18</td>
<td>8±17</td>
</tr>
<tr>
<td></td>
<td><em>Capillaria longipes</em></td>
<td>21±21</td>
<td>62±53</td>
<td>11±17</td>
<td>8±17</td>
</tr>
<tr>
<td>Large intestine</td>
<td><em>Trichuris ovis</em></td>
<td>42±35</td>
<td>15±23</td>
<td>0</td>
<td>8±17</td>
</tr>
<tr>
<td></td>
<td><em>Chabertia ovina</em></td>
<td>163±57</td>
<td>292±220</td>
<td>429±151</td>
<td>215±120</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>All species</td>
<td>25,138±4035</td>
<td>22,168±8703</td>
<td>18,926±4360</td>
<td>13,769±7248</td>
</tr>
</tbody>
</table>
Frances Gulland previously found that *Teladorsagia* spp. monopolised the abomasum of all age classes of sheep that died in the population crash of 1989, comprising around 75% of the burden. Counts of the abomasum nematode species of crash 2002 sheep, however, revealed substantially higher *Trichostrongylus* spp. counts in some individuals and hitherto concealed trends in the burden of the predominant species with age. This discovery prompted an examination of a similar subset of random abomasum samples that had previously been collected in 1999 but not before examined.

Similar opposing distributions with host age were observed for the two abomasum species and no observed significant difference between the crashes of 1999 and 2002 enabled the datasets to be combined (n=124). Figure 10 shows that intensity of *Teladorsagia* spp. increased significantly with host age (p<0.001) as intensity of *Trichostrongylus axei* decreased (p<0.001).

![Figure 10](image)

**Figure 10.** Boxplots showing combined 1999 and 2002 crash worm counts of **a)** *Teladorsagia* spp. and **b)** *Trichostrongylus axei* in relation to host age. For each species there was a strong association with host age but in opposite directions (p<0.001).
In the 2002 crash hosts, the predominant species in the small intestine was *Trichostrongylus vitrinus* which showed highly correlated abundance with *Trichostrongylus axei* across hosts ($r_s=+0.49$; $p<0.001$) (Fig. 11). It is therefore clear that the predominant species in juvenile hosts that died in the population crash of 2002 belonged to the genus *Trichostrongylus*. The worm burden radically changed by the age of two years. Burden of *Trichostrongylus* spp. significantly decreased ($p<0.005$), presumably as a result of a strong acquired immune response to this genus, and the concomitant significant increase of *Teladorsagia* spp. ($p<0.001$) meant that this species predominated in adult sheep. In terms of abundance in the juvenile host, combined *Trichostrongylus* species were on average four times more abundant than *Teladorsagia* spp. In contrast, in the adult host, *Teladorsagia* spp. was around four times more abundant than the combined *Trichostrongylus* species.

Generally, juvenile males had significantly higher mixed gastro-intestinal nematode burden than any other demographic group ($p=0.045$). Of the three predominant species, males had significantly higher burdens of *Trichostrongylus axei* ($p=0.05$) and *Trichostrongylus vitrinus* ($p=0.02$) but there was no significant difference in intensity levels of *Teladorsagia* spp. between the host sexes.

The sex of worms found in the 2002 crash samples was identified. *Trichostrongylus* spp. made up a significantly higher proportion of the strongyle-egg producing female adult community in male hosts than female hosts ($p<0.05$) (Fig. 12). This leads to the conjecture that the higher faecal egg counts generally observed in juvenile male sheep may be as a consequence of higher burdens of *Trichostrongylus* spp. and not *Teladorsagia* spp. as previously believed. Since the eggs of these species cannot be
distinguished microscopically, this point will require molecular investigations in future. *Trichostrongylus* species may also be more likely to contribute to the increased mortality of juvenile males in crashes.

**Figure 12.** Proportions of adult strongyle-egg producing female nematodes in each host sex-age class in 2002 crash sheep.

**Vegetation.**

Mick Crawley.

In August 2004 we had the highest-ever whole-island sheep count, so we expected substantial impacts on vegetation. Based on previous high-density years, we predicted low tussock numbers, high gap cover, low mean sward height, low biomass of palatable grasses and low flowering by grazing-sensitive plant species like *Leontodon autumnalis* (Crawley et al., 2004). As it turned out, we observed extremes of most of these measures. Figure 1 shows the estimated biomass per sheep from the inbye grasslands and its effect on sheep population dynamics:
Figure 13. The relationship between population change between August in year t and August in year t+1 (Delta = ln(N(t+1)/N(t)) and biomass per sheep on the inbye grassland in August of year t (measured as g dry matter per 20 x 20 cm quadrat/whole island count).

Above a threshold of about 0.01 g grass, the sheep population is likely to increase, and the rate of increase is positively correlated with food availability. The odd year out was 1994, when population declined despite reasonably high levels of food availability. In August 2004 we measured just 0.0068 g grass per sheep. There are only two other years when food availability was as low as this in August, and these were the pre-crash years of 1998 and 2001 (0.0069 and 0.0068 g grass per sheep, respectively). Our prediction, therefore, is for a crash in 2005 as severe as the population crashes of 1999 and 2002 (when Delta was -0.74) leading to a predicted whole-island of just 952 in August 2005. We shall see.

In August 2004, the heather had just recovered its condition after the salt-spray lashing it received in summer 2002 (most of the green foliage had been burned off between June and August 2002, and the heather was still in poor condition in August 2003). It will be interesting to see whether the terrible storm of Tuesday 11 January 2005 has an adverse effect on the heather, given that the salt-spray inundation took place outside of the growing season.
Publications

The book (at last)


Research Papers


In Press


ACKNOWLEDGEMENTS

We are grateful to the National Trust for Scotland and to Scottish Natural Heritage for permission to work on St Kilda, and for their assistance in many aspects of the work. The project would not be possible without the generous assistance and support of MOD, QinetiQ and Eurest staff stationed on St Kilda and Benbecula and servicing the island. We are particularly grateful to Natalie McCall, the NTS warden for St. Kilda, especially for counting the number of sheep on the different islands of the St. Kilda group and to Susan Bain the Archaeologist on the island.

We are also grateful for the help of volunteers without whom the fieldwork for 2004 would not have been possible: Eric Allan, Claire Andrews, Lizzie Coleman, Marcus Haber, Neil Hart, Amy Howard, Kenny Kombat, Adrian Leach, Alex Lord, Eric Postma, Gina Prior, Vicky Parker, Anne Schroer, and Matt Tinsley. Thank you.

Grants and studentships from the UK Natural Environment Research Council, the UK Biotechnology and Biological Sciences Research Council, the Wellcome Trust, the Royal Society and the Marie Curie fellowship scheme of the EU support the research on St. Kilda.

APPENDIX A: PERSONNEL CHANGES & SCHEDULE OF WORK

Personnel Changes

Matt Robinson (Edinburgh University) arrived to study the genetics and evolution of the Soay horn polymorphism for his PhD; Tom Ezard (Imperial College) also started his PhD on estimating selection in vertebrate populations; Giacomo Tavecchia (University of Kent) is welcomes back for further development in the statistics of demography.

Bryan Grenfell and Dave Coltman have gone to work in the U.S.A. and Canada respectively; Iain Gordon, Peter Visscher and Allan McRae have all gone to work in Australia; Kate Byrne, Andy Overall, and Barbara Wimmer also left the Project.
Schedule of work on St Kilda

Winter - Spring

From March 12th until May 14th, Jill Pilkington, and four volunteers carried out ten population censuses and tagged and sampled lambs for ongoing genetic studies. 305 lambs were born to 277 ewes; these figures include 28 sets of twins (25 ewes held both lambs, 3 lost one twin). 196 lambs (94 male and 102 female) were caught and tagged; a further 81 lambs died before any tagging attempt. Thirty two animals that had died over winter were found. From April 2nd till June 1st Louisa Tempest and two volunteers carried out suckling watches on ewes and lambs. They also collected and performed faecal egg counts on the control and bolused ewes of the summer catch up 2003.

Summer

Jill Pilkington and two volunteers returned to Hirta on July 13th 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 9th – 21st, was led by Josephine Pemberton, and conducted by a team of 11 other project members and volunteers. 324 sheep were caught and processed, of which 111 were lambs (55 males and 56 females), 67 were yearlings (25 male and 42 females), 43 were adult males, and 103 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 31 Sheep were retagged because of damaged or missing tags. 13 previously untagged sheep (10 lambs, and 3 yearlings) were caught and processed. Jill Pilkington and two volunteer remained on Hirta until 3rd September to complete parasite counts and vegetation monitoring.

Autumn

From October 15th to December 7th Jill Pilkington and two volunteers monitored the mating period, capturing and processing 23 incoming tups, 93 resident tups including recaptures and carrying out ten population censuses. Nine dead sheep were found.
CIRCULATION LIST - (Please advise J.Pilkington of any changes or additions)

Prof. S. Albon  Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Dr. W. Amos  Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Dr. H. Armstrong  Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY.
Ms. S. Bain  NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. D. Bancroft  GPC AG, Lochhamer Str. 29D-82152, Munich, Germany.
Mr. K. Balharry  Loch-na-Leoba, Old Glen Road, Newtonmore, Invernesshire.
Mr. A. Bennett  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr. P. Burman  NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. K. Byrne  Ruthven, Carolsdie, Earlston, TD4 6AL.
Dr. N. Charbonnel  CBGP, Campus de Baillarguet, 34 988 Montferrier s/Lex cedex.
Prof. T. Clutton-Brock  Dept. Zoology, Cambridge Univ., Downing St., CB2 3EJ.
Mr. D. Clark  St. Kilda, c/o QinetiQ, Benbecula, HS7 5LA.
Dr. D. Coltonman  Dept. of Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. J. Fenton  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr. M. Forchhammer  Dept.of Landscape Ecology, NERI, Kalo, DK-8410 Ronde, Denmark.
Prof. I. Gordon  CSIRO, Davies Lab., Univ. Drive, Townsville, QLD 4810, Australia.
Dr. B. Grenfell  Biol. Dept., 208 Mueller Lab., Penn State Univ., PA16802, USA.
Dr. F. Gulland  TMCC, Marin Headlands, Sausalito, CA 94965, USA.
Ms. J. Harden  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.
Dr. A. Hester  Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH.
Dr. O. Jones  Dept. Biology, Imperial College, Silwood Park, Ascot, SL5 7PY.
Dr. L. Kruuk  Inst. Evol. Biol., Edinburgh Univ., West Mains Rd, Edinburgh EH9 3JT.
Prof. I. Kyriazakis  SAC, Bush Estate, Penicuik, Midlothian, EH26 0PW.
Dr. G. Lincoln  MRC Centre for Rep. Biol., 49 Little France Cres., Edinburgh, EH3 9EW.
Dr. J. Lindstrom  IBLS, Univ. of Glasgow, Glasgow, G12 9QQ.
Mr. J. Love  SNH, Stfilligarry, South Uist, HS8 5RS.
Dr. R Luxmoore  NTS, 28 Charlotte Square, Edinburgh, EH2 4DU.
Dr. A. MacColl  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Mr. A. Macdonald  Advisory Services, SNH, Anderson Place, Edinburgh, EH6 5NP.
Mr. D. MacLeannan  SNH, 17 Frances St., Stornoway. Lewis, Outer Hebrides.
Mr. A. Malo  Dept. De Ecologia Evolutiva, CSIC, 28086 Madrid, Spain.
Mr. A. McRae  Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. J. Milner  Hogsokolen i Hedmark, Evenstad, NO2480, Koppang, Norway.
Prof. B. Morgan  Inst. Maths & Stats., Univ. Kent., Canterbury, Kent, CT2 7NF.
Ms. K. Moyes  Dept. Biological Sciences, Imperial College, Silwood Park, Ascot, SL5 7PY.
Mr. S. Murray  c/o SNH, Stfilligarry, South Uist, HS8 5RS.
Dr. A. Overall  Sch. Pharmacy and Biomolecular Sci., Univ. Brighton, BN2 4GJ.
Dr. S. Paterson  School of Biological Sciences, Univ. of Liverpool, L69 7ZB.
Mrs J. Pilkington  Fell House, New Alston, Haydon Bridge, Hexham, NE47 6HR.
Dr. B. Preston  Leahurst Veterinary Field Station, Neston, South Wirral, CH64 7TE.
Mr. A. Robinson  SNH, 17 Frances St., Stornoway. Lewis, Outer Hebrides.
Dr. J. Slate  Dept. of Animal and Plant Sciences, Univ. Sheffield, S10 2TN.
Dr. J. Smith  Dept. Biological Sci., Lancaster University, LA1 4YQ.
Dr. I. Stevenson  Sunadal Data Solutions, 8 West Annandale St, Edinburgh, EH7 4JU.
Dr. G. Tavecchia  Inst. Maths & Stats., Univ. Kent., Canterbury, Kent, CT2 7NF.
Miss L. Tempest  IBS, Stirling Univ., Stirling, FK9 4LA.
Dr. P. Visscher  Queensland Inst. Med. Res., PO Royal Brisbane Hospital, Q4029, Australia.
Dr. J. Watson  SNH, Fraser Darling House, 9 Culduthel Road, IV2 4AG.
Dr. K. Wilson  Dept. of Biological Sciences, Lancaster University, LA1 4YQ.
Dr. B. Wimmer  Grunlandweg 16, 85375 Neufahrn, Germany.
Mr. S. Wiseman  NTS, Balnain House, 40 Huntly St., Inverness, IV3 5HR.