ST. KILDA SOAY SHEEP PROJECT: ANNUAL REPORT 2017

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POPULATION OVERVIEW 2
REPORTS ON COMPONENT STUDIES 4
Vegetation *4*
Survival costs of reproduction are mediated by parasite infection *8*
Selection and evolution of lamb size and pregnancy *10*
Pedigree based estimation of reproductive values *13*
Investigating the genetic basis of vitamin D status *15*
Immunosenescence *17*
Maternal transfer of parasite-specific antibodies and fitness in neonates. *19*
PUBLICATIONS. 21
ACKNOWLEDGEMENTS.. 21
APPENDIX A: PERSONNEL NEWS & SCHEDULE OF WORK 22

Population Overview

The sheep population on Hirta entered 2017 at a moderate level and there was relatively high mortality over winter with 88 tagged animals being found dead with in the study area. Lambing began on the 7th of April with 74.7% of lambs born surviving (Fig. 1).

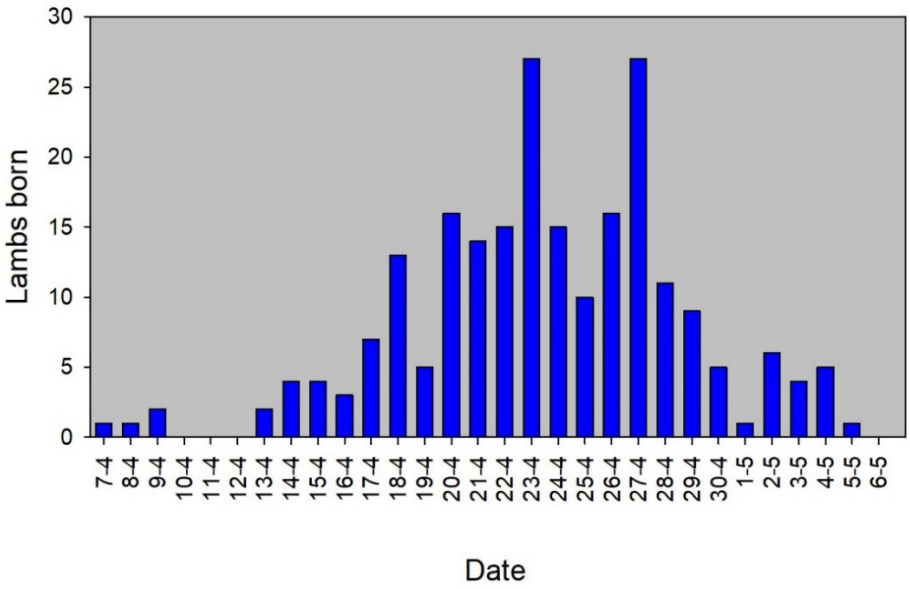

Figure 1. *The temporal distribution of lamb births during 2017.*

In December 2017, 711 tagged sheep were believed to be alive on Hirta, of which 528 regularly used the study area, an increase of 18.9% using the study area since the previous year. The age distribution of the population is shown in Figure 2 and changes in sheep numbers in the study area over time are shown in Figure 3.

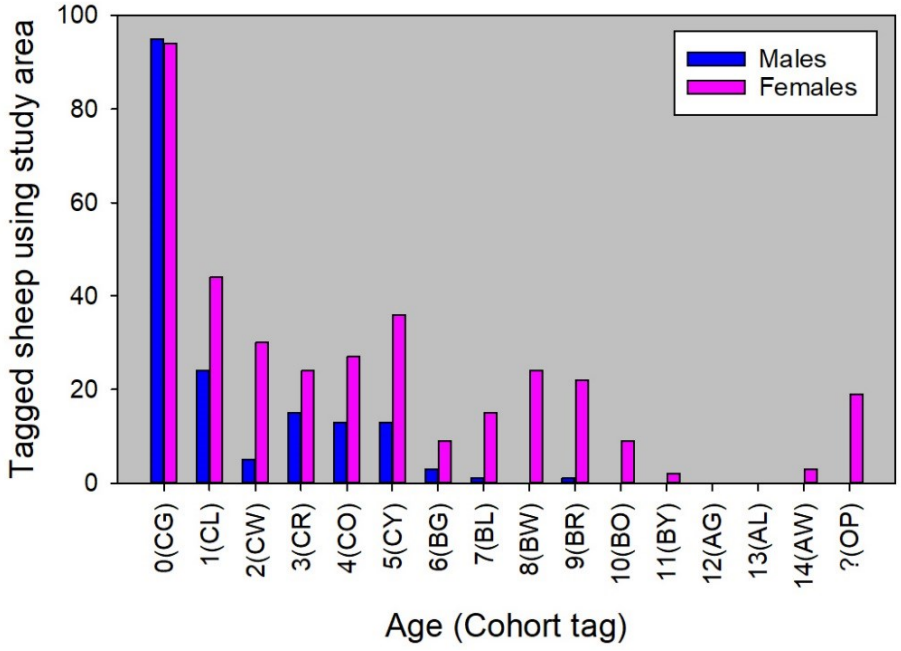

Figure 2. *Age distribution of tagged Soay sheep presumed to be alive at the end of 2017.*

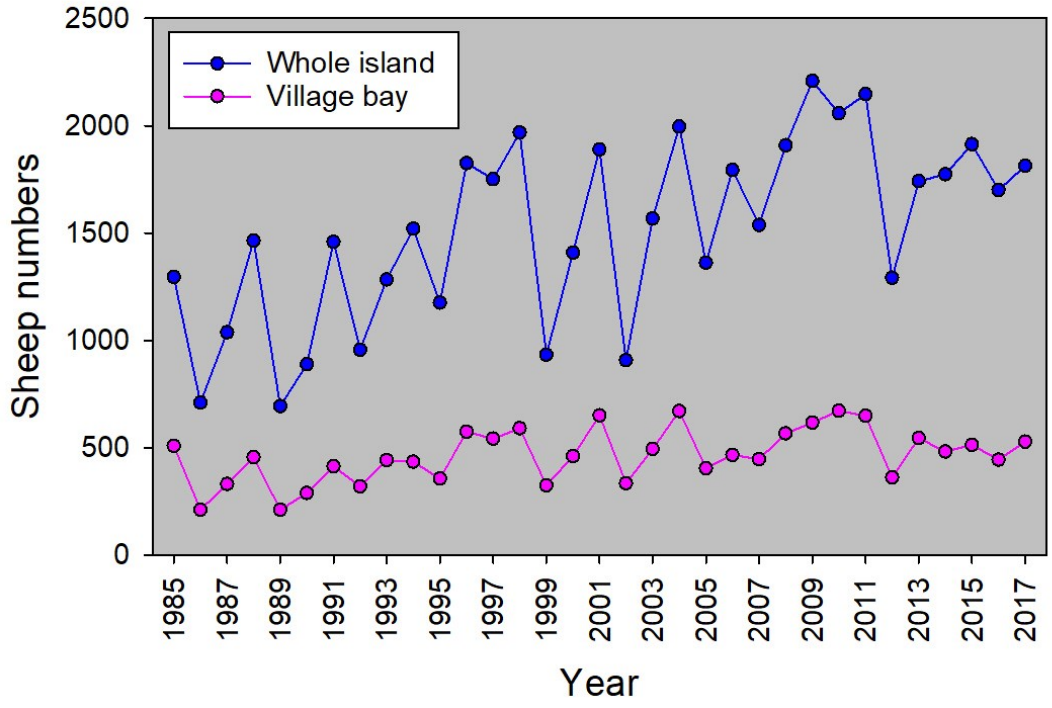

Figure 3. *The number of sheep counted on the whole island and the number of tagged sheep regularly using the study area since 1985.*

One whole-island count yielded 1813 tagged and untagged sheep, with the details displayed in Table 1. The total population had increased by 6.6% since summer 2016 when it was estimated as 1701. This gives a delta (calculated as ln (N_{t+1}/N_t)) of 0.064. The whole island count is also shown in Figure 3.

Table 1. *Demographic and geographic distribution of sheep observed during the count of Hirta on August 13th 2017. Coat colours are DW = dark wild, DS = dark self, LW = light wild, and LS = light self.*

Location	**Females**				**Males**				**Lambs**	**Total**
	DW	**DS**	**LW**	**LS**	**DW**	**DS**	**LW**	**LS**		
Conachair/Oiseval	174	9	44	3	38	1	7	0	162	**438**
Mullach Bi / Cambir	246	9	77	5	25	1	3	0	281	**643**
Ruaival/Village	260	12	86	4	65	1	28	0	276	**732**
Total	**680**	**26**	**207**	**12**	128	**3**	**38**	**0**	**719**	**1813**

Vegetation.

Mick Crawley.

August 2017 saw the completion of a 25 year study into the effects of selective grazing by Soay sheep on the botanical composition of St Kilda's principal plant communities.

The rationale was that the Soay sheep of St Kilda represent an ideal study system for the study of plant-herbivore dynamics. The sheep have no competitors (no deer, rabbits or voles) and no predators (no foxes or eagles), so the herbivore population was likely to be food-limited. Likewise, all of the herbivore impacts on the plant community could be attributed unequivocally to the sheep. If we could not understand plant-herbivore dynamics in a community as simple as St Kilda, then what hope would we have with more complex ecosystems?

The first message to emerge from the study was that plant species' identity is hugely important. Palatable species like the grass *Festuca rubra*, soon replace most or all of the other plant species when sheep are permanently excluded, as they are on the ungrazed turf roofs of the tallest and steepest-sided cleits, and on sheep-free islands like Dun. There is clearly a trade-off between palatability and growth rate. Plants like *Holcus lanatus* that invest in defence grow more slowly, so in the absence of grazing these species are outcompeted by plants like *Festuca rubra* that invest in high growth rates. The high-growth, high-palatability species have high tissue concentrations of nutrients like nitrogen, but low concentrations of toxins and digestibility-reducing compounds such as tannins.

The outcome of competition between the plant species is mediated by selective grazing by the sheep. Their preference for high growth-rate plants like *Festuca rubra* reduces the competitive ability of these potential dominants and allows less palatable, more slow-growing species like *Holcus lanatus* to coexist. It is clear that grazing by Soay sheep has a strong positive effect on plant species richness in plant communities on St Kilda: the ungrazed cleit roofs have 2 or 3 species in a 20x20cm quadrat, whereas the grazed turf typically has 10 or more species in a similar area.

We have known for many years that the fluctuating population of Soay sheep imposes radically different grazing pressures on the plant communities in different years. The sheep population typically increases rapidly from low densities, then after 2 or 3 years reaches a population density that is too high to be supported by the amount of food available during the winter. At this point the sheep population suffers high (occasionally very high) levels of mortality, with most of the deaths concentrated in the youngest and oldest age classes, and with males typically suffering a higher death rate than females. From time to time, the sheep population declines by more than 60% between January and April (these are the years that we refer to as 'population crashes'). Intriguingly, however, the death rate never gets much higher than this, no matter how harsh the weather or how little there is to eat.

This 'stability despite extreme fluctuation in population size' derives from the age-structure and the sex-structure of the sheep population, and from the resilience of the sward to chronically high levels of grazing.

The population dynamics of the sheep are driven by over-compensating density dependent mortality, brought about as a result of intraspecific competition for food during the winter in years when sheep numbers are high. The long-term stability of the sheep population derives from the fact that this competition for food is highly asymmetric in its consequences for the

animals. All of the animals may be hungry, but it is the weaker or sicker animals that are disproportionately likely to die. In years when the population crashes, virtually all of the lambs will die, as will many elderly animals along with a good number of mature males. In marked contrast, the 2 - 5 year old female sheep are much less likely to die. They have reached full adult size, and have had time to build up fat reserves that are sufficient to carry them through the hungry months of winter. This means that even when there is mass mortality of young and old animals, the population does not go extinct, because these adult females are pregnant with roughly equal numbers of male and female lambs to re-establish the age structure in the following year. It is this asymmetry (known as 'contest competition' in the jargon of population dynamics) that allows long-term persistence of the sheep despite huge fluctuations in population size.

What about the plants? Why does such protracted, heavy grazing not lead to their demise? There are at least two answers to this. First, the plants that are most palatable to the sheep are able to grow big enough during years of low sheep numbers that they can lay down sufficient reserves of carbohydrate and protein to see them through the years when sheep numbers are high. The less palatable plant species survive because they get just enough of a competitive advantage during the years of heavy grazing pressure to prevent competitive exclusion by the more palatable species (as we observe on Dun and on ungrazed cleit roofs). Second, the inaccessible, underground parts of the plants represent a strongly stabilizing influence on the system as a whole. The existence of this ungrazeable reserve of vegetative buds and stored food reserves means that the sheep cannot eliminate the plants, no matter how high the grazing pressure becomes.

You might imagine that because sheep numbers are food-limited, then the plant community must be 'over-grazed' in some sense, and that long-term declines in plant productivity (and hence in sheep numbers) must be inevitable. We have found no evidence of this. If anything, the sheep population has been getting larger, although the last 5 years have bucked the trend of increasing sheep numbers that we reported in 2012 (at that time, the whole island count was going up by more than 30 extra sheep per year on average). The 2013-2017 sheep population has been substantially lower, and has fluctuated rather gently about the long-term average. It is plausible that the increase in sheep population from 1985 to 2012 was driven by increased plant productivity, probably associated with rising winter temperatures.

There are big differences in grazing impacts between years, with different effects in different seasons. The biggest visual effects in winter, for example, are differences in the density of tussocks. There are large and numerous tussocks in winters following summers with low sheep numbers, but tussocks are scarce in years when sheep are most abundant and almost absent in crash years (Fig. 4). Tussocks are bad news for the sheep in winter because they are full of indigestible dead organic matter which reduces the average quality of each bite of food. In summer, the biggest visual differences between years are in the density of flowers. The summer pasture is a haze of yellow in low sheep years, but uniformly grassy green when sheep are abundant. There is a small group of plant species whose flowering increases immediately following reductions in grazing pressure. This was neatly illustrated in summer 2017 after the erection of sheep-proof fences around the construction site on the QinetiQ base. Within a few weeks of the fences being completed, the sward was golden yellow with the flowers of autumn hawkbit (*Leontodon autumnalis*) and meadow buttercup (*Ranunculus acris*), studded with white clover (*Trifolium repens*) and daisies (*Bellis perennis*). The grasses respond more slowly to reductions in grazing pressure, and there was no enhanced flowering on the base by the end of August. Some species (like *Festuca rubra*) are so palatable that they have never been observed to flower in the grazed sward in 25 years, no matter how low

sheep numbers have become. The less palatable grasses (like *Holcus lanatus* and *Anthoxanthum odoratum*) flower profusely in the year after sheep numbers have been low (Fig. 5). Other plants are more mysterious in their flowering behaviour, showing little or no relationship between flowering and grazing intensity, past or present. What we don't know is how these fluctuations in flowering lead to differences in the rate of establishment of new plants from seed. Virtually all of the plant species in the grazed communities on St Kilda are perennials, and individual clones are probably very long-lived, surviving for decades rather than just a few years. Seedlings are rarely seen on St Kilda for any of the perennial plant species, and we still have much to learn about the evolutionary aspects of sheep grazing on plant population genetics.

In summary, we have documented coexistence within a multi-species plant community, despite intense selective grazing. Competitive exclusion is prevented by a combination of niche differences between the plants (mainly differences in palatability and growth rate), combined with strongly fluctuating grazing pressure exerted by our single, food-limited herbivore population. You can find more detail about the responses of the plant species to grazing in The Flora of St Kilda, which was published to coincide with the completion of this study (Crawley M.J., 2017, *The Flora of St Kilda*, Hebridean Naturalist Supplement 1: 1-61).

Figure 4a. *Tussocks are most abundant in late winter following years of low sheep numbers. This is Signals Meadow in March 2015.*

Figure 4b. *Tussocks are rare in winters following years of high sheep numbers. This is Signals Meadow in the crash year of March 2002.*

Figure 5a. *The maximum* Holcus lanatus *flowering-stem density was recorded in August 2013. The low grazing pressure in this year is reflected by the lack of any conspicuous difference in biomass or flower-stem density outside (grazed) and inside the pyramid (ungrazed for more than 4 months). The sheep exclosure had been moved to this position in March 2013.*

Figure 5b. *The minimum flower-stem density of* Holcus lanatus *was recorded in August 2017. There is hardly a grass flower to be seen in this view of West Fields. Robin Pakeman (on the left) is cutting a tussock sample and Paul Tinsley-Marshall (right) is cutting a gap.*

Survival costs of reproduction are mediated by parasite infection.

Jessica Leivesley, Luc Bussière, Jill Pilkington & Adam Hayward.

A central principle of evolutionary life-history theory is that individuals command a finite pool of resources (e.g. protein, energy, time), meaning that they face trade-offs: for example, protein invested in reproduction cannot be invested in self-maintenance. The theory of parasite-mediated reproductive costs predicts that reproduction should be associated with suppressed defence against parasites due to resource allocation trade-offs between reproduction and defence, reducing future survival. Reproduction is often associated with reduced parasite resistance in wild populations, but to support the hypothesis of parasite-mediated reproductive costs, we must also demonstrate that reduced parasite resistance is associated with reduced fitness.

We quantified the pathways linking reproductive investment, parasite burden and survival using data collected from the Soay sheep in 1988-2008. The effects of reproduction on gastrointestinal strongyle nematode faecal egg count (FEC) have been well characterized in domestic sheep: reproduction is associated with an increase in FEC known as the peri-parturient rise (PPR), due to the large resource investment required for lactation.

First, we analysed how reproductive investment influenced the PPR in the Soay sheep. Individuals experienced an increase in FEC during the middle of the season, followed by a subsequent decline. Further, reproductive investment affected the trajectory of the PPR: younger animals had higher FEC than older animals; adult females which reproduced had

higher FEC than non-reproducing adults; adult females who suckled their lambs to weaning had higher FEC than females whose lamb died before weaning (Fig. 6). Overall, we found evidence for a cost of reproduction in the form of increased parasite burden.

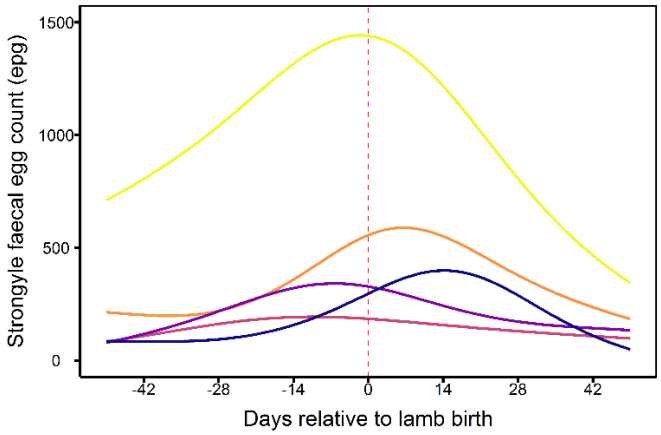

Figure 6. *Model estimates showing predicted changes in strongyle FEC across the lambing season. Day 0 is the day on which a female gave birth; for males and non-reproducing females, day 0 is the average lamb birth day for a given year. Lambs (yellow) and yearlings (orange) have very high faecal egg counts. Among adults, females which suckled their lamb(s) to weaning (dark blue) had higher peak FEC than those whose lamb(s) died before weaning (purple), which had higher peak FEC than females and males which did not reproduce (pink).*

Next, we assessed the survival consequences of reproduction through direct effects and indirect effects mediated by infection and body weight, using structural equation models (SEMs). Our final SEM showed that reproduction in April had a strong, direct, positive effect on probability of death over the subsequent winter (Fig. 7), supporting a direct cost of reproduction (path: Reproduction → Winter Death). Our SEM also showed that reproduction influenced survival through a number of other pathways, many of which were mediated by strongyle FEC. For example, reproduction was associated with higher FEC during lambing, which was associated with lower August body weight, which was linked to higher mortality (path: Reproduction → Lambing FEC → Body weight → Winter Death). Many other paths were also supported, as described in Figure 7.

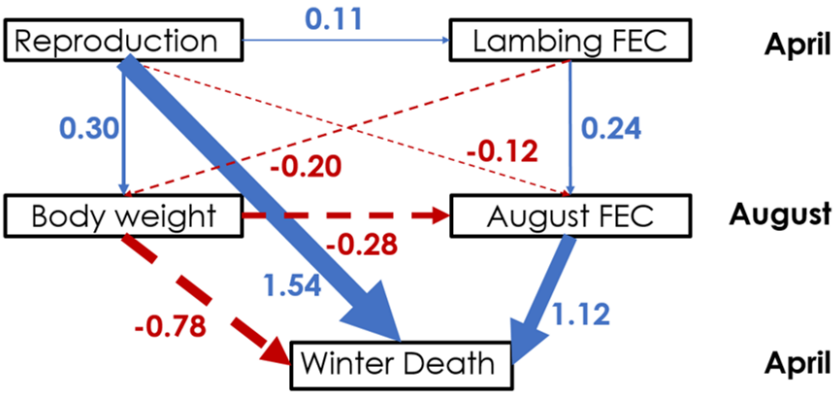

Figure 7. *Illustration of SEM determining associations between reproduction and subsequent survival. Blue lines indicate positive associations; red lines show negative associations; numbers represent standardized path coefficients.*

We can draw the following conclusions from these analyses. First, all individuals experience an increase and decrease in FEC during the lambing season. This is due to the effects of deteriorating and then improving general body condition at the end of a harsh winter; the increasing activity of strongyles in early spring; the increased shedding of strongyle eggs onto pasture by infected animals. Second, adult females investing more in reproduction, and particularly those with lambs surviving to weaning, had a higher peak FEC which occurred later. This points to the importance of investment in lactation driving both the magnitude and timing of the increase in FEC during the spring. Third, the greater FEC in females investing more in reproduction came at a cost to survival during the subsequent winter. Along with a direct effect of reproduction, this meant that, overall, females investing more in reproduction in spring were more likely to die over the following winter.

Selection and evolution of lamb size and pregnancy.

Maria Joao Janeiro, Dylan Childs, Josephine Pemberton, Jill Pilkington & Michael Morrissey.

The genetics underlying the evolution of growth are not yet fully understood in Soay sheep. While theory predicts evolution towards larger body size in this population, field observations show no evidence of that, even suggesting that the opposite is taking place, i.e. there is no change or even a decline. A contribution to clarifying the dynamics of body size might lie in a disadvantageous yet persisting behaviour – pregnancy during the first year of life. Around 40% of the ewes get pregnant as lambs and those who do so are much more likely to die during their first year.

We found that this pregnancy in the first year of life is highly dependent on body mass, being more likely to occur in larger ewe lambs (Fig. 8). Such an association between lamb size and lamb pregnancy results in a trade-off between survival and fecundity. Being larger is associated with higher rates of first-year survival and total offspring production over a lifetime (lifetime rearing success, LReS, defined as the number of offspring successfully reared at least until November 1), but on the other hand being pregnant as a lamb is

associated with lower first-year survival (Fig. 9). This trade-off is central to understanding selection of body size in Soay sheep ewe lambs, but can only play a role in evolutionary change if lamb body mass and lamb pregnancy are correlated at a genetic level. We found this to be the case, by estimating an additive genetic correlation of over 0.6 (95% Credible Interval: 0.24; 0.90).

We developed a model of selection that combines separate analyses of the effects of mass on pregnancy and first-year survival, and also on subsequent rearing success of those that survived their first year. The model shows that selection favouring large body size is considerably weaker than what would be estimated if the size-pregnancy trade-off in the first year of life did not exist. We also showed that around half the selection in lamb body mass occurs later in life, rather than in lambs, due to fecundity at later ages.

Our work shows how a trade-off between viability and fecundity selection in body mass diminishes the advantage of being larger as a ewe lamb, partly explaining why Soay sheep are not evolving larger body size as fast as some models predict. Our work also raises the question of why lamb pregnancy, having been established as a maladaptive behaviour, still persists in the Soay sheep population of Hirta.

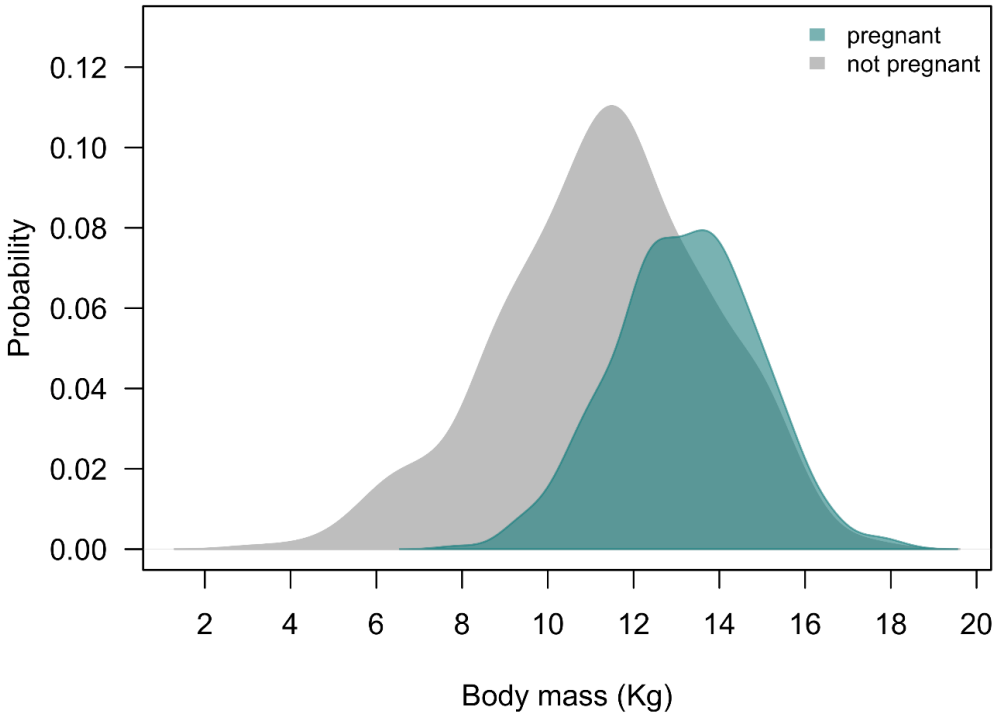

Figure 8. *Probability that a ewe lamb becomes pregnant based on her body mass the previous August, showing that the ewe lambs that become pregnant tend to be amongst the largest.*

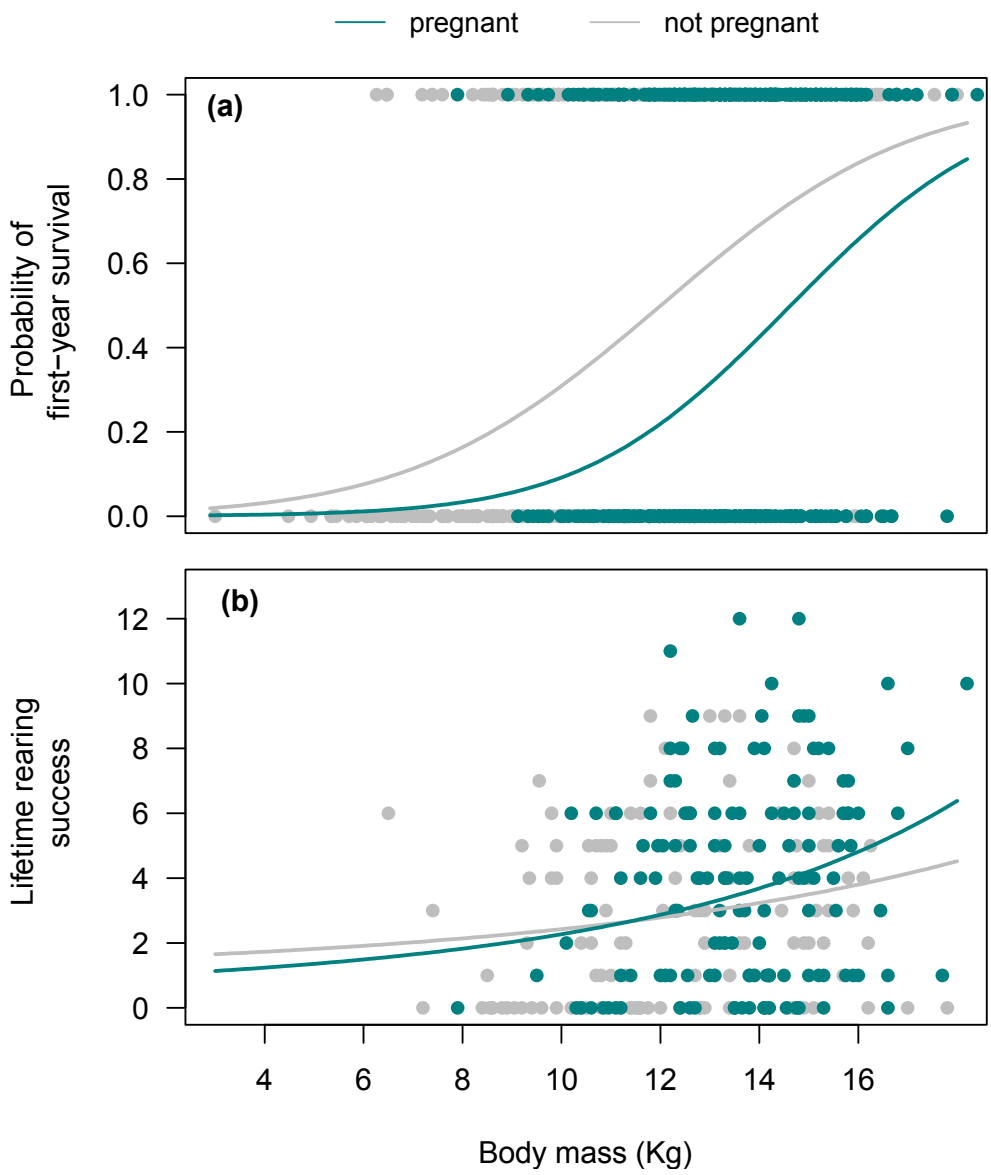

Figure 9. *Probability of first-year survival* ***(a)*** *and lifetime rearing success, LReS,* ***(b)*** *in Soay sheep ewe lambs as a function of lamb body mass the preceding August and lamb pregnancy status. Dots correspond to observed data and curves to model predictions. First-year survival was modelled using a binomial regression with logit link function, whereas a Poisson regression with exponential link function was fitted to LReS data. In both cases, population density, and maternal age were included as covariates (intercepts and slopes were estimated for both), as well as cohort and maternal ID as random effects.*

Pedigree Based Estimation of Reproductive Values.

Darren Hunter & Michael Morrissey.

It is often necessary to quantify the evolutionary success of a given individual or group of individuals (e.g., a cohort). One way to assess this is through the expected representation of their genes in the future population. This value at a point in the distant future is referred to as the reproductive value. While this concept is well understood in the theoretical literature, it can be difficult to estimate in wild populations. Often, the success of an individual cannot be predicted accurately from the overall success of a cohort (Fig. 10). Long-term data sets such as that assembled by the Soay Sheep Project along with high quality pedigree data allow the development of new approaches to understanding how this contribution will change over time for both individuals and cohorts.

Typical methods for analysing fitness often struggle to take into account fluctuations in the age structure and population size, which are common attributes of wild populations. The availability of a pedigree allows calculation of fitness estimates that takes into account the survival and fecundity of individuals as well as their descendants. By focussing on a single cohort at a time we can take subsections of the pedigree that relate these focal individuals to their descendants. Doing this for different time points after their birth allows us to calculate their expected genetic contribution at each of these times. This allows us to visualise how this contribution changes through time and we can then make observations about how quickly these values appear to be stabilising and if they appear to be doing so sufficiently in the timeframes that we have available to us.

In most cases, the genetic contributions calculated from the pedigree, for both individuals and cohorts, settle to relatively constant values within reasonably short time periods. Figure 11 shows the individuals born into the 1986 cohort as an example, while Figure 12 shows the cohort-level estimated reproductive values for the cohorts from 1985 to 1989. In the first years after birth these values can oscillate quite dramatically, but then quickly settle down. This happens especially quickly, in around 10 years, for the cohort level expected genetic contributions. There is a great deal of variation in the settled ultimate genetic contributions to the population, both within and between cohorts. The fact that we do start to see the expected genetic contributions stabilising within the timeframe of the Soay sheep study suggests that calculation of reproductive values from pedigrees could become more commonplace for long term datasets. The type of information estimated using this method is likely to be valuable to ecologists, demographers and quantitative geneticists and we expect this method will be useful for answering questions across many subject areas. However, it is worth noting that there is a large amount of variation in the individual estimated genetic contributions and that there are individuals born early in the study, who are unusually successful, and whose contribution to the population still appears to be changing (Fig. 11). The causes of the long-term performance of these lineages remains to be investigated.

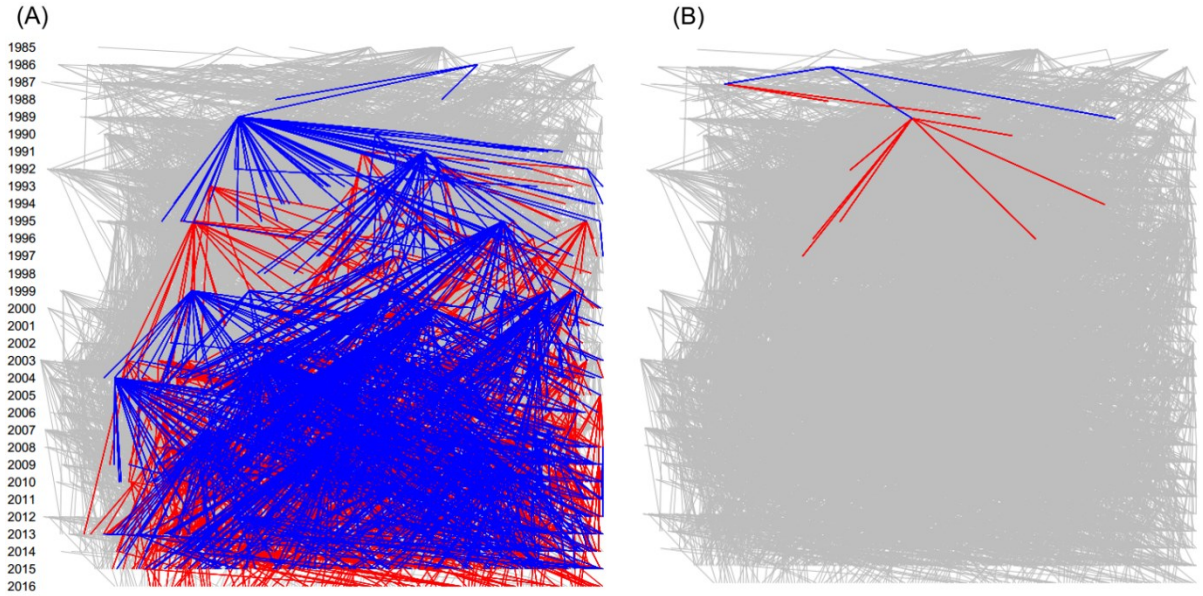

Figure 10. *Pedigrees of two separate male individuals from the 1986 cohort, one of which* ***(A)*** *produced many descendants and has made a large contribution to the current population and the other* ***(B)*** *which has no descendants after their grand offspring and therefore this individual has made no contribution to the current population.*

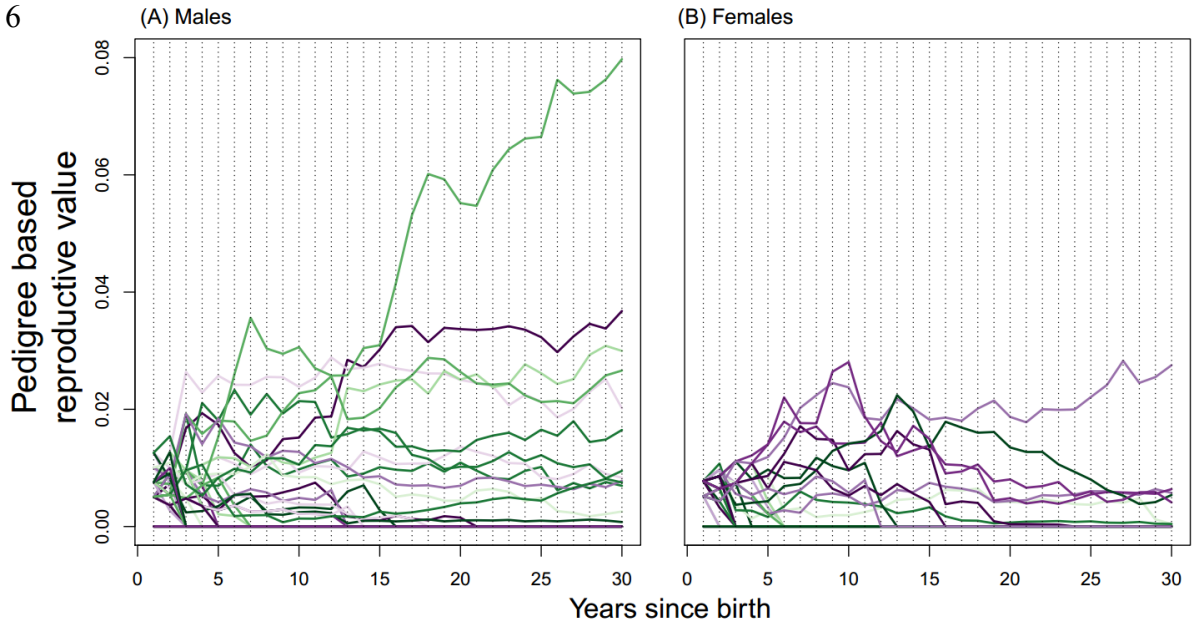

Figure 11. *Individual pedigree-based genetic contribution of males* ***(A)*** *and females* ***(B)*** *born in 1986. Each line represents a separate individual born that year. Every individual from the cohort is plotted as a separate line to show their pedigree-based expected genetic contribution estimate each year after their birth.*

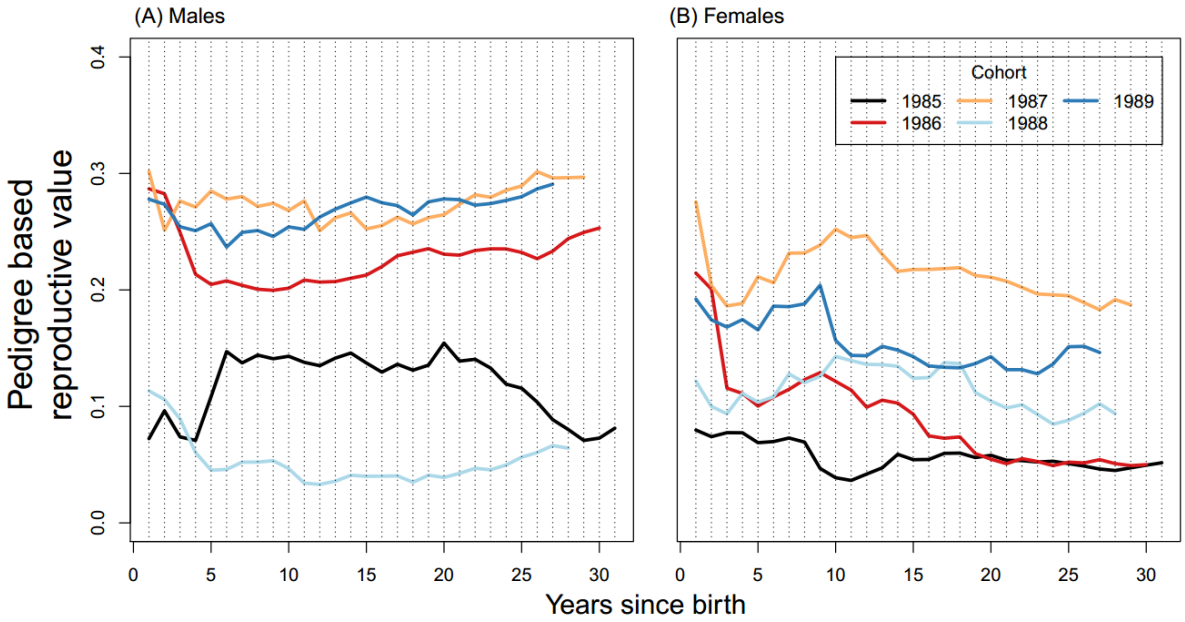

Figure 12. *Total cohort pedigree based expected genetic contribution for males* ***(A)*** *and females* ***(B)****. Each line represents a separate cohort from 1985 to1989. The pedigree-based expected genetic contribution for that cohort every following year is plotted, with 1985 having the longest line with 31 years of data.*

Investigating the genetic basis of vitamin D status.

Alex Sparks, Susan Johnston, Ian Handel, Kathryn Watt, Jill Pilkington, Josephine Pemberton, Dan Nussey & Richard Mellanby.

Vitamin D plays a critical role in the maintenance of skeletal health and there is growing interest in the role of vitamin D plays in the health of non-skeletal tissues. Our previous work had shown a dramatic association between vitamin D status, as measured by the major vitamin D metabolite 25 hydroxyvitamin D (25(OH)D) and fecundity in Soay sheep. Given the increasing evidence that vitamin D may play an important role in non-skeletal health in both wild sheep and humans, the aim of this project was to explore the genetic basis of vitamin D status in the Soay sheep population.

A total of 1,457 August plasma samples from 918 sheep over a 6 year period (2011-2016) were assayed for levels of the two vitamin D metabolites $25(OH)D_2$ and $25(OH)D_3$. We found that vitamin D levels were repeatable across an individual's lifetime, with 41% and 35% of the variation in $25(OH)D_2$ and $25(OH)D_3$ levels explained by individual identity, i.e. variation between individuals. By looking at the similarity of vitamin D levels among relatives using the population pedigree, we were able to estimate the amount of individual variation in vitamin D levels explained by genetic effects. A significant proportion of the variation in the two vitamin D metabolites was explained by genetic differences between individuals. Heritabilities of $25(OH)D_2$ and $25(OH)D_3$ across all ages were 0.23±0.05 and 0.26±0.05 respectively. In addition, there was evidence for a weak but positive genetic correlation between the two metabolites (r_g=0.33±0.13). However, heritabilities cannot identify the underlying genetic variants associated with vitamin D levels. So we next tested

whether any individual single nucleotide polymorphism (SNP) of ~40,000 across the genome was associated with the two vitamin D metabolites using a method called a genome wide association study (GWAS). However, after a correction for multiple testing, no SNPs were associated with either 25(OH)D_2 or 25(OH)D_3 levels. Our results suggest that vitamin D levels are fairly stable across time within individuals, and this is in part due to genetic variation between individuals. However, a GWAS approach did not detect any genetic variants associated with vitamin D levels. This suggests that vitamin D levels are under polygenic control and are influenced by many loci each of which have small effects on vitamin D levels. Further work will address how vitamin D levels change with age and whether vitamin D levels are associated with fitness components such as survival and rearing success.

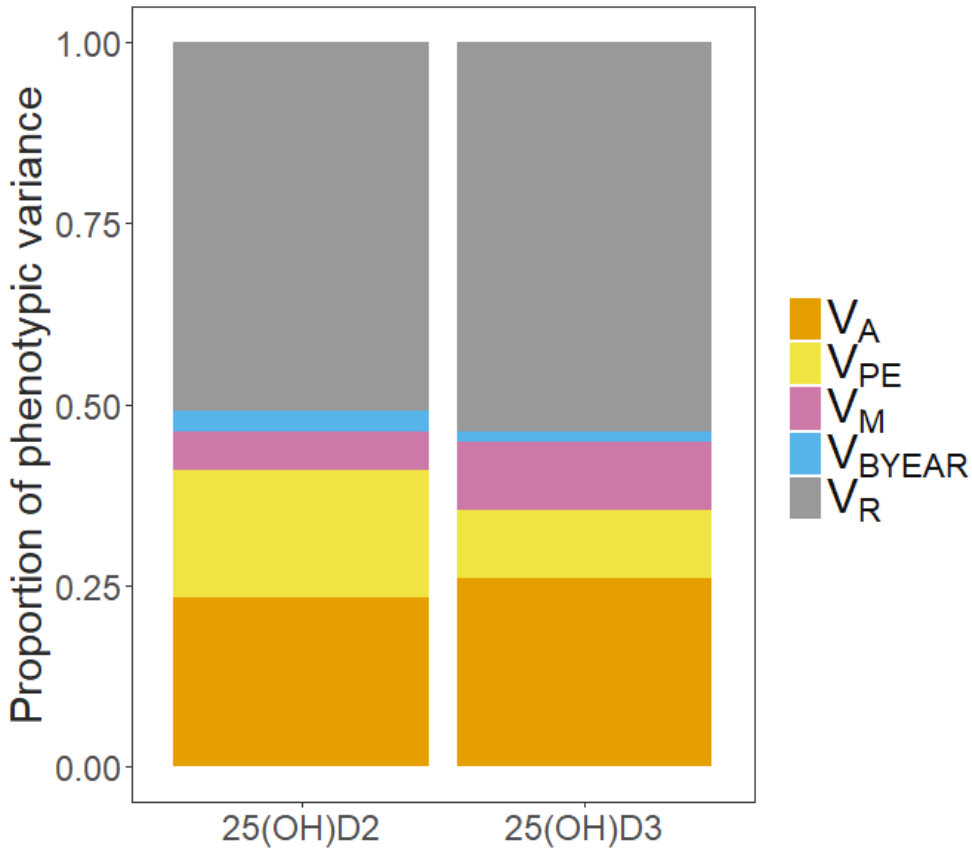

Figure 13. *The proportion of phenotypic variance explained by different random effects in animal models for two vitamin D metabolite measures (25(OH)D_2 and 25(OH)D_3) across all ages in Soay sheep. Random effects include the following variance components: additive genetic (V_A), permanent environment (V_{PE}), maternal ID (V_M), birth year (V_{BYEAR}) and the residual (unexplained) error (V_R). Repeatability is the sum of the proportion of phenotypic variance explained by additive genetic and permanent environment components.*

Immunosenescence.

Hannah Froy, Alex Sparks, Florian Bach, Kathryn Watt, Josephine Pemberton, Tom McNeilly & Dan Nussey.

Declines in reproductive performance and survival probability with age are increasingly observed in wild vertebrate populations, and this demographic senescence has been shown to have important consequences for population dynamics. However, the physiological processes that drive these declines are much less well understood. A key question is the extent to which mechanisms identified in humans and laboratory studies also shape the ageing patterns of wild animals, which are subject to highly variable environments, food limitation, and constant parasite challenge. Declining immune function, or immunosenescence, is an important component of the ageing process in humans, and there is increasing evidence that it could also play an important role in the wild. However, most studies examining age-related variation in immune function in natural populations are cross-sectional. Because immune response is likely to vary between individuals, and this may have consequences for survival, it can be difficult to know exactly what is going on within individuals as they age. In addition, information on individual fitness components is required to understand the consequences of potential declines in immune function, and whether these could contribute to demographic senescence.

The Soay sheep project offers an excellent opportunity to study aspects of immune function across the entire natural lifespan. We used archived plasma samples collected from adult sheep during the August catch 1990 – 2015 to determine how parasite-specific antibody levels vary with age (n=1866 samples from 643 individuals). We measured antibodies specific to *Teladorsagia circumcincta*, an important and highly prevalent gastrointestinal nematode which infects the sheep. Previous work in the Soay sheep has shown that levels of anti-*T.circ* IgG are positively associated with survival over crash winters. We found that levels of IgG were highly repeatable within individuals, meaning that those with relatively high levels of IgG tended to have consistently high levels throughout adulthood (60% of the variance in IgG was explained by individual identity). However, we found that levels of IgG declined as individuals approached death (Fig. 14), which is indicative of immunosenescence. Levels of IgG were negatively correlated with faecal egg count (Fig. 15), and within-individual declines in IgG were associated with increases in faecal egg count between years, suggesting the senescent decline has consequences for parasite burden. Finally, we found that levels of IgG were positively associated with survival probability, which is consistent with previous studies. However, this new longitudinal dataset allowed us to decompose this relationship into the effect of between-individual differences in IgG, and the effect of within-individual changes in IgG. We found that the positive association between IgG and survival was driven by within-individual deviance in IgG (Fig. 16), rather than the average level of IgG across adulthood. So what matters for survival isn't whether an individual has consistently high levels of IgG, but whether their IgG levels are relatively high or low compared to their average. This suggests that the senescent decline in IgG has important consequences for fitness, and could therefore be an important driver of demographic senescence in this population.

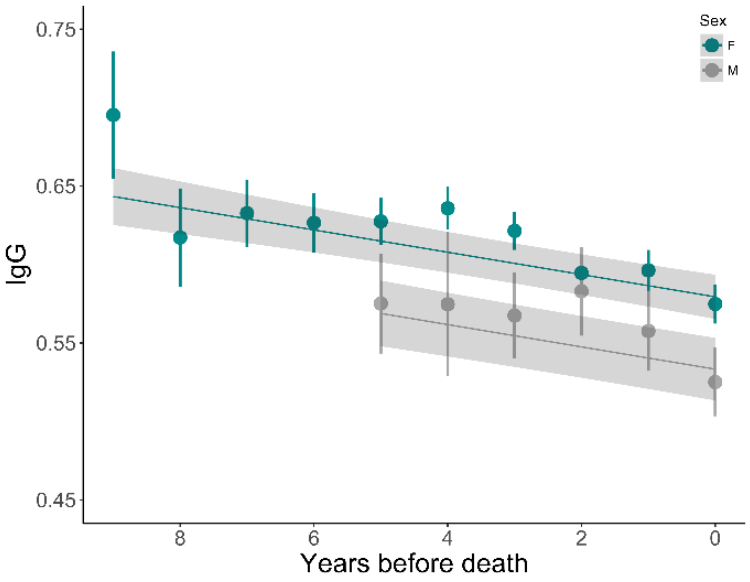

Figure 14. *Levels of anti-*T.circ *IgG declined as individuals approach death in sheep aged 3+ years. Points show raw data means, and lines show predictions from a linear mixed effects model (including individual, year and plate as random intercept terms), both with associated standard errors. The relationship is shown separately for females (green) and males (grey).*

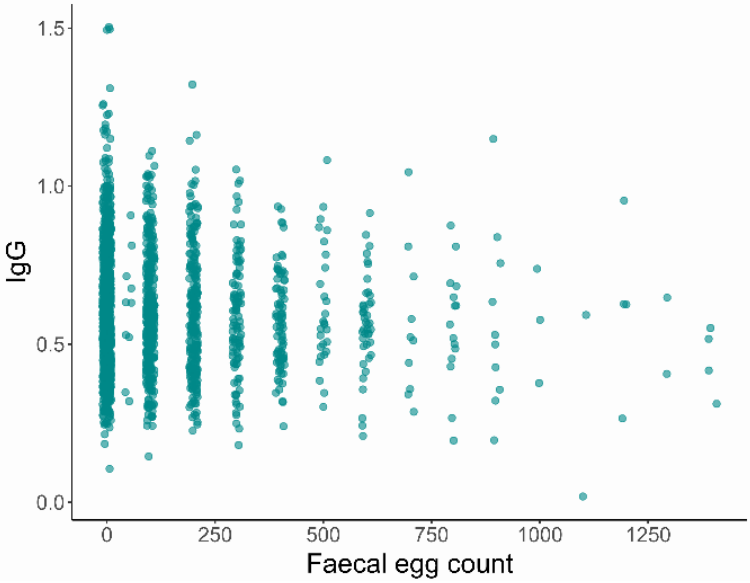

Figure 15. *Levels of anti-*T.circ *IgG were negatively associated with faecal egg count. Points show raw data.*

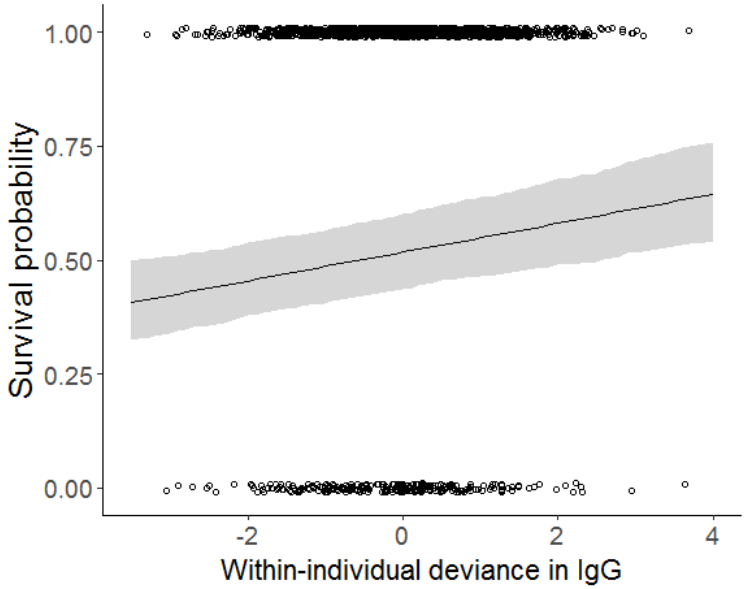

Figure 16. *Overwinter survival probability was positively associated with within-individual deviance in anti-*T.circ *IgG. Points show raw data, and line shows predictions from a generalised mixed effects model (including age, sex, weight, and the interaction between age and sex as fixed effects, and individual and year as random intercept terms) and associated credible intervals.*

Maternal transfer of parasite-specific antibodies and fitness in neonates.

Alex Sparks, Kathryn Watt, Susan Johnston, Tom McNeilly & Dan Nussey.

Maternal antibodies transferred from mother to offspring in early life are crucial in providing neonates with protection from parasites and pathogens while their own immune system is developing. In the wild, it is expected that variation in maternally-derived antibodies in neonates will have important consequences for the health and fitness of offspring in early life. However, although maternal antibodies have been widely acknowledged to be associated with offspring growth and survival in domestic ruminants and laboratory animals, evidence for benefits for offspring in the wild are rarer and may be limited by lack of immunological reagents and knowledge of natural parasite infections. The Soay sheep population on St Kilda is an ideal study system for studying the role of maternal antibody transfer in the wild. Unlike humans, there is no prenatal transfer of antibodies from mother to foetus across the placenta in ruminants so ingestion of colostrum (antibody-rich milk produced close to birth) is crucial for passive immune transfer. In addition, neonatal ruminants are born with a 'leaky' gut which allows them to take up antibodies into their bloodstream for about 24-36 hours after birth. The majority of Soay lambs born within the study area are caught and blood sampled within a few days. As neonatal ruminants are unable to synthesise their own antibodies for several weeks, we know that all antibodies in plasma samples taken from lambs close to birth are maternally-derived. This provides an excellent opportunity to study the causes and consequences of variation in maternally-derived antibody levels in neonatal Soay sheep.

We measured antibodies to a common helminth parasite (*Teladorsagia circumcincta*) for three different isotypes (IgA, IgE and IgG) in 3,491 plasma samples from neonatal lambs that

were caught in the lambing seasons of 1990-2015. We found that lambs caught closer to birth had higher maternally-derived antibody levels, consistent with gut closure and subsequent clearance of antibodies from the bloodstream. We also found that prime age mothers were more likely to have lambs with higher antibody levels. There was also evidence that variation in maternally-derived antibody levels was associated with later offspring health and fitness. Neonates with higher maternally-derived IgG levels were more likely to survive to, and be heavier at, 4 months old (Fig. 17). In addition, lambs with higher maternally-derived IgG levels had lower strongyle faecal egg counts (an indirect measure of parasite burden) at 4 months old and were more likely to survive their first winter (Fig. 17). This provides evidence that variation in maternally-derived antibodies has consequences for early growth and survival of Soay lambs independent of other early life traits that are frequently measured. This study also provides rare evidence of both short and long-term fitness benefits of maternal antibodies in the wild. Further work will investigate the role of maternal antibody levels on later immune development, as well as looking into the genetic basis of maternal antibody levels.

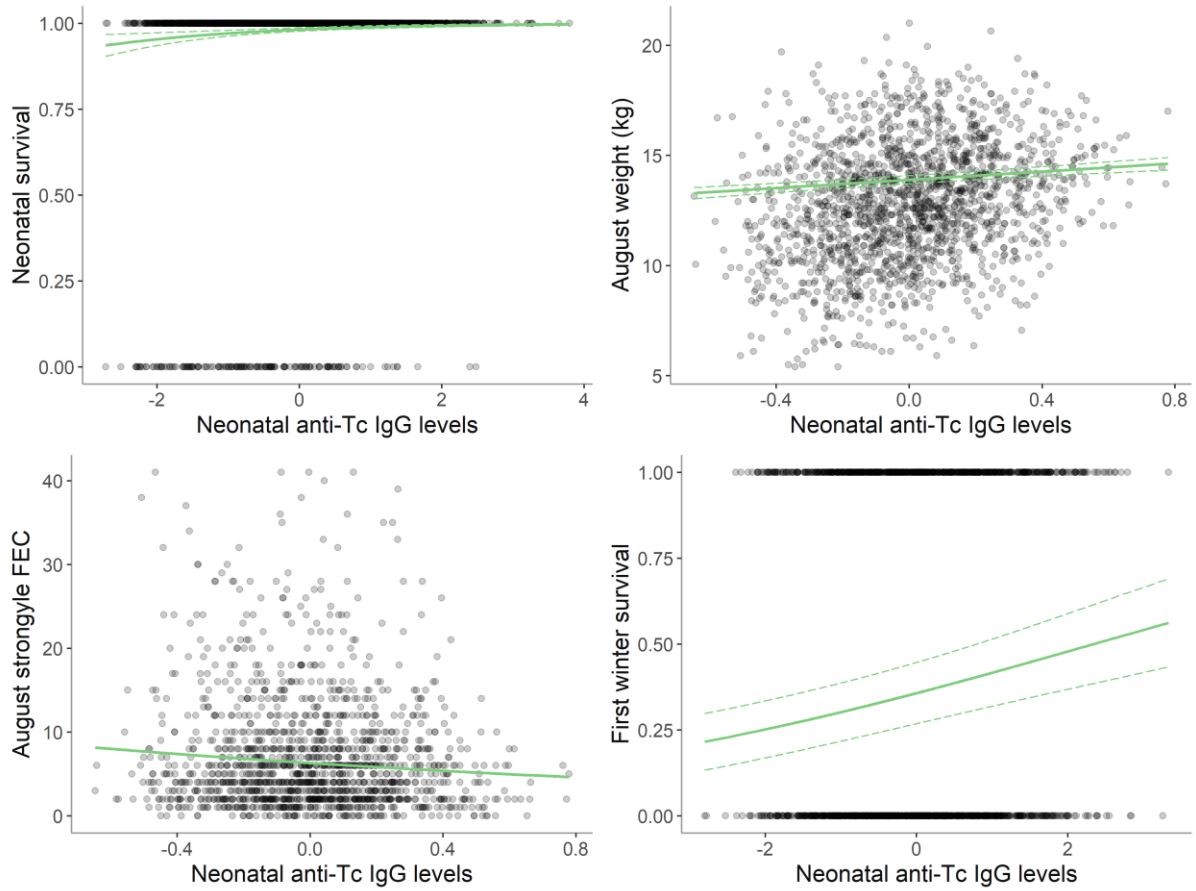

Figure 17. *Associations between neonatal anti-Tc IgG levels and neonatal survival, August weight, August strongyle faecal egg count (FEC) and first winter survival. Plots show raw data with LMM or GLMM predictions and standard errors estimated for singleton male lambs with average values for all other continuous fixed effects in the model.*

PUBLICATIONS

Hayward, A., Pemberton, J.M., Berenos, C., Wilson, A.J., Pilkington, J.G. and Kruuk, L.E., 2017. Evidence for selection-by-environment but not genotype-by-environment interactions for fitness-related traits in a wild mammal population. *Genetics*, pp.genetics-300498. doi: 10.1534/genetics.117.300498

Regan, C.E., Pilkington, J.G. and Smiseth, P.T., 2017. Female Soay sheep do not adjust their maternal care behaviour to the quality of their home range. *Behavioral Ecology*, *28*(4), pp. 962-973. doi: 10.1093/beheco/arx033

Wilkie, H., Riggio, V., Matika, O., Nicol, L., Watt, K.A., Sinclair, R., Sparks, A.M., Nussey, D.H., Pemberton, J.M., Houston, R.D. and Hopkins, J., 2017. A candidate gene approach to study nematode resistance traits in naturally infected sheep. *Veterinary Parasitology, 243,* pp. 71-74. doi: 10.1016/j.vetpar.2017.06.010

Froy, H., Bird, E.J., Wilbourn, R.V., Fairlie, J., Underwood, S.L., Salvo-Chirnside, E., Pilkington, J.G., Bérénos, C., Pemberton, J.M. and Nussey, D.H., 2017. No evidence for parental age effects on offspring leukocyte telomere length in free-living Soay sheep. *Scientific reports*, *7*, p.9991. doi:10.1038/s41598-017-09861-3

Garnier, R., Bento, A.I., Hansen, C., Pilkington, J.G., Pemberton, J.M. and Graham, A.L., 2017. Physiological proteins in resource-limited herbivores experiencing a population die-off. *The Science of Nature*, *104*(7-8), p.68. doi: 10.1007/s00114-017-1490-4

Caudron, Q., Garnier, R., Pilkington, J.G., Watt, K.A., Hansen, C., Grenfell, B.T., Aboellail, T. and Graham, A.L. 2017. Robust extraction of quantitative structural information from high-variance histological images of livers from necropsied Soay sheep. *Royal Society Open Science*, *4*(7), p.170111. doi: 10.1098/rsos.170111

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APPENDIX A: PERSONNEL NEWS & SCHEDULE OF WORK

Personnel News

Mick Crawley retired from fieldwork on St Kilda after 25 years of collecting and sorting botanical samples. Robin Pakeman will be continuing the botanical research.

Charlotte Regan, Kara Dicks and Alex Sparks (all Edinburgh) completed and defended their PhDs successfully; they have all gone on to further research employment.

Wei Huang joined the project to continue research on the evolution of the Major Histocompatibility Complex (MHC) for his PhD with Josephine Pemberton (Edinburgh).

Schedule of work on St Kilda

Winter - Spring

From March 17th until May 12th, Jill Pilkington, Xavier Bal and Louise Christensen carried out ten population censuses and tagged and sampled lambs, with assistance during the peak of lambing from Michael Morrissey and Darren Hunter. 225 lambs were born to 209 ewes; these figures include 16 sets of twins (14 ewes held both lambs, 1 ewe held one lamb and lost one and 1 ewe lost both lambs). 168 lambs (87 male and 81 female) were caught and tagged; a further 57 lambs died before any tagging attempt. Mick Crawley and two assistants collected vegetation data.

Summer

Jill Pilkington, Sam Collins and Fiona Backhouse returned to Hirta on July 18th to carry out ten population censuses, conduct mortality searches (yielding 6 tagged dead animals), and prepare for the main catch-up of study area sheep. The catch-up took place from August 9th – 23rd and was conducted by a team of 12 additional project members and volunteers. 274 sheep were caught and processed, of which 110 were lambs (52 males and 58 females), 36 were yearlings (14 males and 22 females), 15 were adult males, and 113 were adult females. All animals were weighed and measured to monitor growth, and sampled for parasite and genetic analyses. 30 sheep were retagged because of damaged or missing tags. 18 previously untagged lambs, 4 yearling and 4 adults 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 20th to December 8th Jill Pilkington, Xavier Bal and Emma Moreland carried out ten population censuses, monitored the mating period, capturing and processing 15 incoming tups, 21 resident tups and 12 resident ewes. 34 previously darted, non-resident tups were seen in the study area during this rut. 7 dead sheep were found.