## NEWS AND VIEWS

### PERSPECTIVE

# Bad coat, ripped genes: cryptic selection on coat colour varies with ontogeny in Soay sheep

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A fundamental goal for evolutionary biologists is to connect the processes of natural selection and genetic drift with the maintenance of genetic variation in the wild. There are now scores of examples in which mapping phenotypes to genotypes has identified the molecular basis of traits in natural populations, but documenting the fitness consequences of these genes has proven more elusive. A long-term study of several thousand unmanaged sheep on the remote Scottish island of Hirta in the St. Kilda archipelago provides a unique opportunity to make these connections between genotype, phenotype and fitness. Since 1985, there has been a steady decline in the frequency of sheep with a rare uniform ('self'-) pigmentation pattern as opposed to the more common wild-type pattern of dark upper body and pale belly. In this issue of *Molecular Ecology*, Gratten *et al.* (2012) link variation at the agouti signalling protein (ASIP) gene with 25 years of pedigree information and individual fitness data to investigate the contributions of natural selection and genetic drift to this decline. Consistent with the decrease in frequency of self-type sheep, Gratten *et al.* demonstrate that recessive genetic variants at ASIP are associated with reduced lifetime fitness. But surprisingly, these variants show the opposite trend to the self-type phenotype by increasing throughout the study period. This paradoxical result occurs because heterozygotes that harbour the majority of self-type alleles have increased in frequency. This pattern would not have been detectable if only phenotype had been monitored because the pigmentation pattern of heterozygotes is indistinguishable from that of wild-type homozygotes. The study provides an excellent example of the importance of measuring selection at both the phenotypic and genetic level, and demonstrates how long-term pedigrees can be used to link alleles to phenotypes and ultimately to fitness.

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### Genotype, phenotype and fitness

To be able to predict adaptive evolution, it is necessary to know both the strength of natural selection and the genetic basis of affected traits. Accordingly, a diversity of approaches using a variety of data has been utilized to try to link phenotypic targets of selection with their causative genetic architecture. Most commonly, selection is measured on phenotypes, and then genetic mapping is used to identify the genomic regions affecting the traits under selection. Alternatively, statistical signatures of historical selection are identified in regions of the genome affecting putatively adaptive traits. Rarely, however, is selection directly and simultaneously measured on both genotypes and phenotypes. Because the connections between genotype and phenotype are rarely straightforward, estimating selection at these two levels is unlikely to provide redundant information. Instead, discrepancy between patterns of selection on phenotypes and causal genes can help to identify previously unknown mechanisms of selection and clarify the confounding roles of linkage disequilibrium, pleiotropy and epistasis (Barrett & Hoekstra 2011). Unfortunately, obtaining direct estimates of adaptive evolutionary processes at the genetic and phenotypic level in natural populations is no easy task. Most studies are too short to record responses to selection across generations, and those that do often lack molecular information. Gratten *et al.* (2012) circumvent these issues by using the long-term Soay sheep data set from the island of Hirta – a remarkable resource that provides multigenerational records of phenotype, genotype, survival and reproductive success on thousands of individually tagged sheep.

### Pigmentation variation in Hirta Soay sheep

The horned Soay sheep arrived before the Vikings when humans first settled in the St. Kilda archipelago of northwest Scotland. The sheep are derived from the earliest domestic flocks that spread across Europe in the Bronze Age. The particular study population on Hirta island was founded in 1932 with the introduction of about a 100 sheep from the neighbouring island of Soay and has never been managed by humans. Since 1985, this population has been studied on an individual basis, with newborn lambs caught each spring and tagged, measured and sampled for genetic analysis. This data collection has resulted in an impressive pedigree containing over 7000 individual records spanning 10 generations. Records show that over the last 25 years,

most sheep on the island have been black or brown with a pale coloured belly ('wild-type' phenotype). However, there is a rare (∼5%) phenotype that is uniformly, or 'self'-coloured (Clutton-Brock & Pemberton 2004) (Fig. 1A). This rarer phenotype is recessive and has shown a significant decline in frequency as records began. A molecular investigation by the same group has recently shown that the self-phenotype is associated with homozygosity of haplotypes with any of three distinct mutations in the agouti signalling protein (ASIP) gene (Gratten *et al.* 2010). The objective of this new study was to determine whether changes in the frequency of the self-phenotype and underlying ASIP alleles were due to natural selection. Using gene-dropping simulations, Gratten *et al.* (2012) compared the observed temporal trends in the frequency of self-type sheep, ASIP alleles and genotypes, with the distribution of regression slopes expected in the absence of selection, controlling for known pedigree relationships, sampling effects and changes in population size.

## Ontogenetic trade-offs aid heterozygotes

Despite a significant decline in self-type sheep, the frequency of self-type ASIP alleles has increased rapidly since 1990 (Fig. 1B). This unexpected result is all the more surprising because the self-type allele confers reduced juvenile survival (s coefficient ∼0.42). The reason can be understood by analysing the dynamics of genotypes rather than alleles. Heterozygote genotypes, which carry the majority of self-type alleles in the population, have sharply increased in frequency. This increase occurs at the expense of both self and wild-type homozygotes, and gene-dropping simulations show that surplus of heterozygotes exceeds expectations for genetic drift. Because self-type alleles increase in frequency while residing in heterozygous sheep that have the same pigmentation pattern as homozygous wild-type sheep, the success of the alleles is not detectable at the phenotypic level. Indeed, from phenotypic data alone, one might make the prediction that self-type alleles are in decline. But why have heterozygotes increased in frequency? Interestingly, the excess of heterozygotes may be due to the sexual prowess of self-type homozygotes, which have higher reproductive success than other genotypes (s = ∼0.24). Matings between self-type sheep are rare because of their low frequency, so most of the time self-type sheep will mate with either a wild-type homozygote or heterozygote, which produces either 100% or 50% heterozygous offspring, respectively. As such, although self-type sheep have reduced viability, their advantages in reproductive success fuel a heterozygote surplus and in turn cause the self-allele to increase in frequency. Note that this dynamic is inherently frequency dependent because self-type sheep will only produce predominantly heterozygous offspring while homozygous wild-type genotypes are common. The results highlight the complexity of predicting long-term evolutionary trajectories of putatively adaptive genes, even in cases like the Soay sheep of Hirta, where exceptional life history and pedigree information have been collected over multiple generations.

## Moving forward

The agouti locus has been well studied, in part because it has been used to measure mutation rates (Green 1989) and because several alleles are clinically relevant. But despite the wealth of knowledge about the structure and developmental dynamics of the gene, it is unclear what functional mechanisms are responsible for its effects on juvenile survival and adult reproductive success in Soay sheep. The association between ASIP and fitness may not be due to the effects of the gene on pigmentation, but rather because of pleiotropic effects on some other, as yet unidentified trait. Alternatively, ASIP may be in linkage disequilibrium with one or more other fitness-related loci. Unfortunately, this unresolved dichotomy between the effects of pleiotropy and linkage is a common end point for many studies of the genetics of adaptation. The use of next-generation sequencing to provide high-density markers (or sequence) around ASIP should help to discriminate between these two possibilities and allow for independent estimates of selection in increasingly narrow genomic windows (Burke *et al.* 2010). Of course, no gene operates in isolation, and
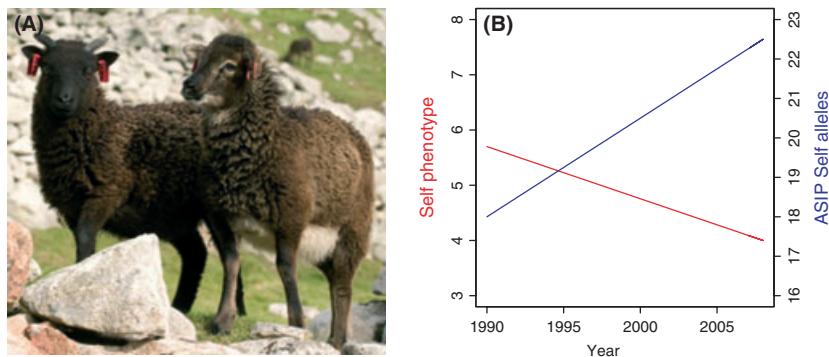


**Fig. 1** (A) Self- (left) and wild-type (right) coat pattern in Soay sheep (photograph Arpat Ozgul). (B) Population frequency of self-type Soay sheep (red line, linear regression 1985–2008: slope = −0.07%/year, adj. $R^2$ = 0.267, $P$ = 0.006) and recessive self-type agouti signalling protein alleles (blue line, linear regression 1990–2008: slope = 0.22%/year, adj. $R^2$ = 0.620, $P$ = 3.8 × 10⁻⁵).

the use of genomic methods should also allow the 'gene-centric' focus on ASIP to be expanded to consider the emergent fitness effects of larger numbers of interacting loci. The combination of next-generation sequencing, long-term pedigree information and individual-based fitness estimates will be a powerful approach for predicting future microevolutionary patterns in this one-of-a-kind study system.

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