LETTER

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Life history trade-offs at a single locus maintain sexually selected genetic variation

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Sexual selection, through intra-male competition or female choice, is assumed to be a source of strong and sustained directional selection in the wild[1,2]. In the presence of such strong directional selection, alleles enhancing a particular trait are predicted to become fixed within a population, leading to a decrease in the underlying genetic variation[3]. However, there is often considerable genetic variation underlying sexually selected traits in wild populations, and consequently, this phenomenon has become a long-discussed issue in the field of evolutionary biology[1,4,5]. In wild Soay sheep, large horns confer an advantage in strong intra-sexual competition, yet males show an inherited polymorphism for horn type and have substantial genetic variation in their horn size[6]. Here we show that most genetic variation in this trait is maintained by a trade-off between natural and sexual selection at a single gene, relaxin-like receptor 2 (*RXFP2*). We found that an allele conferring larger horns, Ho^{+}, is associated with higher reproductive success, whereas a smaller horn allele, Ho^{P}, confers increased survival, resulting in a net effect of overdominance (that is, heterozygote advantage) for fitness at *RXFP2*. The nature of this trade-off is simple relative to commonly proposed explanations for the maintenance of sexually selected traits, such as genic capture[7,8] ('good genes') and sexually antagonistic selection[5,9]. Our results demonstrate that by identifying the genetic architecture of trait variation, we can determine the principal mechanisms maintaining genetic variation in traits under strong selection and explain apparently counter-evolutionary observations.

The persistence of genetic variation in traits under sustained sexual selection is a fundamental paradox in evolutionary biology, for which several explanations have been proposed. First, sexually dimorphic characters could be an honest signal of male quality or condition, in which the best-condition males develop the largest traits[7,8]. Under this 'genic capture' model, many loci contribute to male condition, creating a large mutational target for the sexually selected trait. As a result, genetic variation will persist despite strong directional selection[7]. Second, variation may be maintained by genetic trade-offs that constrain evolution of the focal trait. For example, sexually selected traits often exceed the point at which they would be optimal for survival[10], indicating that trade-offs exist between sexual and non-sexual fitness. A variant on this model is intra-locus sexual conflict, driven by sexually antagonistic selection[5,9]; in this scenario, alleles that increase male fitness are associated with decreased female fitness (and vice versa).

Testing and disentangling which of the theories explains empirical patterns remains difficult owing to the relatively limited knowledge of the genetic architecture (that is, the number of genes and the magnitude of their effects) of relevant traits[11,12], as there are remarkably few systems where the genes responsible for sexually selected trait variation are known. However, the advent of affordable genomic technologies now provides an unparalleled opportunity to identify genes responsible for fitness-related variation in wild populations[13–15].

One wild population where the genetic architecture of a sexually selected trait has been characterized is the Soay sheep of St Kilda (*Ovis aries*), a population of primitive domestic sheep that has existed completely unmanaged for around 4,000 years and has been intensively studied since 1985. During the mating season (rut), there is strong competition between males for access to oestrous females. Most males develop normal horns, but around 13% develop vestigial horns (scurs) conferring reduced reproductive success[16] (Fig. 1). The horn size of normal-horned males is positively correlated with mating success[17], yet there is substantial heritable variation underlying this trait ($h^2 = 0.37$)[6]. Females develop much smaller horns, and are either normal-horned (32%), scurred (40%), or 'polled' (lacking horns or scurs, 28%; Supplementary Fig. 1). Recent studies have revealed that a single gene, relaxin-like receptor 2 (*RXFP2*), explains most of the genetic variation in horn morphology in Soay sheep[6] and domestic sheep[18,19]. Two *RXFP2* alleles, Ho^{+} and Ho^{P}, have been identified in Soay sheep: Ho^{+} confers larger, normal horns, whereas Ho^{P} confers smaller horns, with around half of $Ho^{P}Ho^{P}$ males developing scurs[6] (Fig. 1). Furthermore, *RXFP2* contributes ~76% of the additive genetic variation in horn size in normal-horned males, including normal-horned $Ho^{P}Ho^{P}$ males[6] (Fig. 1 and Supplementary Note 1). This discovery provides a critical opportunity to understand the relative importance of sexual and natural selection in maintaining genetic variation underlying horn development.

Figure 1 | Horn morphology variation with *RXFP2* genotype. Examples of adult male horn morphology with their corresponding *RXFP2* genotypes. **a**, Four-year-old normal-horned $Ho^{+}Ho^{+}$. **b**, Five-year-old normal-horned $Ho^{+}Ho^{P}$. **c**, Five-year-old normal-horned $Ho^{P}Ho^{P}$. **d**, Three-year-old scurred $Ho^{P}Ho^{P}$.

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We used genetic and phenotypic data from 1,750 sheep sampled over a 21-year period to understand how genetic variation at *RXFP2* is maintained and to determine whether microevolution of horns is occurring. To achieve this, we: (1) examined differences in annual reproductive success, survival and overall fitness between *RXFP2* genotypes; (2) determined selection coefficients and equilibrium frequencies of the two *RXFP2* alleles; and (3) assessed whether temporal changes in allele frequency were consistent with balancing selection maintaining genetic variation at *RXFP2*.

The *RXFP2* genotype was associated with annual reproductive success in adult males, as Ho^PHo^P males had significantly lower reproductive success than both Ho^+Ho^+ and Ho^+Ho^P males (Markov chain Monte Carlo (MCMC) generalized linear mixed model: $P_{MCMC} = 0.004$; Fig. 2a). The *RXFP2* genotype was also associated with male annual survival, such that Ho^+Ho^+ individuals had lower survival than both Ho^+Ho^P and Ho^PHo^P individuals (MCMC generalized linear mixed model: $P_{MCMC} = 0.006$ and 0.010, respectively; Fig. 2b). When reproductive success and survival were combined into an overall fitness metric, Ho^+Ho^P males had higher fitness than both Ho^+Ho^+ and Ho^PHo^P individuals (MCMC generalized linear mixed model: $P_{MCMC} = 0.042$ and 0.036, respectively; Fig. 2c). In females, there was no relationship between *RXFP2* genotype and survival, reproductive success or overall fitness (MCMC generalized linear mixed models: $P_{MCMC} > 0.05$). Full results for all models are given in Supplementary Tables 1 and 2.

Analyses of overall fitness of all sheep indicated that variation at *RXFP2* is maintained by overdominance, resulting in an equilibrium frequency of 0.529 for the Ho^P allele (bootstrap 95% confidence interval: 0.284–0.793). This is driven by each homozygous genotype being associated with reduced fitness (that is, Ho^+Ho^+ with reduced survival and Ho^PHo^P with reduced reproductive success), which has resulted in a net effect of highest overall fitness in Ho^+Ho^P individuals (Supplementary Table 3). The products of effective population size and selection coefficients were considerably greater than one for annual reproductive success, survival and overall fitness, confirming that selection is probably more important than genetic drift in maintaining genetic variation at *RXFP2* within this population[20,21] (Supplementary Table 3).

Although the frequency of the Ho^P allele increased over the study period (linear regression: $b = 0.426\%$ per year, adjusted $R^2 = 0.653$, $P = 1.74 \times 10^{-5}$; Supplementary Fig. 2), simulations of the expected change in frequency given the pedigree indicated that this increase is not greater than would be expected by chance (gene-drop simulation, one-tailed $P = 0.109$), further showing that the change in frequency is unlikely to be driven by directional selection. The observations that selection coefficients are strong enough to exceed the effects of drift, yet temporal changes in allele frequency are no greater than expected by drift, may seem contradictory. However, because selection coefficients on reproduction and survival are in opposite directions (from the context of a homozygous genotype) with a net effect of overdominance, marked temporal shifts in allele frequencies are not expected to occur. In fact, allele frequencies seem to have converged on and possibly stabilized at something close to the equilibrium frequency (Supplementary Fig. 2). Furthermore, analysis of *RXFP2* haplotype sharing between Soay sheep and other breeds of *Ovis aries* indicates that the polymorphism may have been present in Soay sheep throughout much of their long history on St Kilda (Supplementary Note 2). Therefore, as a result of the large contribution of *RXFP2* to horn growth, genetic variation in horn morphology is maintained by a trade-off between reproductive success (favouring the large-horn allele Ho^+) and survival (favouring the small-horn allele Ho^P) in male Soay sheep.

The analyses presented here have allowed an empirical test of the key hypotheses proposed to explain genetic variation in sexually selected traits. As a single locus explains nearly all of the genetic variation in horn type and size[6] (see Supplementary Note 1), the genic capture hypothesis can be discounted. More plausible explanations involving major loci, particularly intra-locus sexually antagonistic selection, were also ruled out as *RXFP2* is associated with fitness components in males, but not in females. Instead, variation at *RXFP2* seems to be maintained by a simple trade-off in males, where only heterozygous males (Ho^+Ho^P) are uncompromised by poor survival or low reproductive success; different horn types in females are merely a genetic consequence of the trade-off in males. Investigation of selection coefficients supports the idea that there is a net effect of overdominance, or heterozygote advantage, at this locus in Soay sheep. Despite being one of the most intuitive explanations for the maintenance of genetic variation, convincing examples of overdominance remain rare[22,23]. Therefore, selection on *RXFP2* in male Soay sheep may be an additional entry to what remains a short list of compelling cases of heterozygote advantage[24].

The exact mechanism by which the *RXFP2* genotype influences variation in male survival and reproductive success in Soay sheep is unknown. However, it does seem to be mediated by the effect of *RXFP2* on horns, as scurred Ho^PHo^P males have lower reproductive success than normal-horned Ho^PHo^P males (Supplementary Note 3). Fitness variation at *RXFP2* may be due to differences in energy expenditure by the three genotypes in relation to their horn size in males, particularly during the rut. Larger, normal-horned males can spend 50% of their time holding individual female consorts, but spend less time feeding and must defend their consort against harassment by juveniles and competition from other dominant males[25,26]. Subordinate males, including juveniles and smaller horned males, can spend just 5% of their time in consorts and instead mate opportunistically, actively seeking undefended females. Therefore, as *RXFP2* genotype directly affects horn phenotype, this may determine whether males are likely to engage in a mating strategy with high annual reproductive success but low survival, or a strategy with low annual reproductive success but high survival.

Ultimately, the findings in this study advance our understanding of the maintenance of genetic variance in a trait under natural and sexual

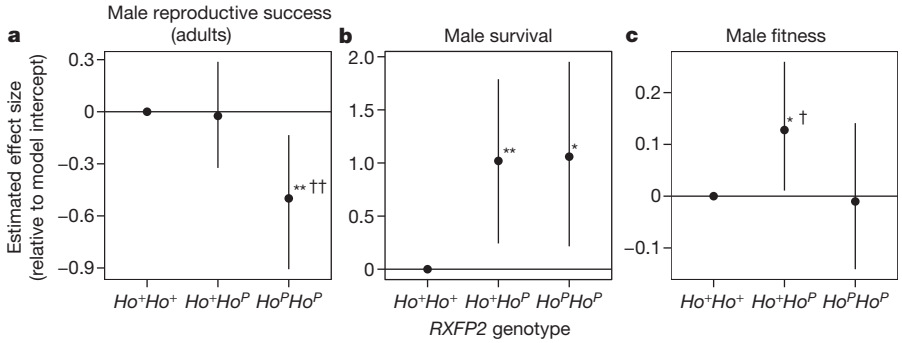

Figure 2 | Annual fitness variation and *RXFP2* genotype. **a**, Reproductive success in adult males ($n = 640$). **b**, Survival in all males ($n = 1,243$). **c**, Overall fitness in all males ($n = 1,204$). Effect sizes were estimated from the posterior mode of a MCMC generalized linear mixed model and are given relative to the model intercept at Ho^+Ho^+. Vertical bars indicate the 95% credible interval. The single asterisk and double asterisk indicate a significant difference from the intercept at Ho^+Ho^+ at $P_{MCMC} = 0.05$ and 0.01, respectively; single and double daggers indicate the same for the model intercept at Ho^+Ho^P.

selection in a wild population. The ability to identify the gene responsible for sexually selected variation has made it possible to distinguish between competing hypotheses for the persistence of horn variation and investigate an evolutionary response (or stasis). Further studies that dissect the genetic architecture of sexually selected traits in other systems will make it possible to establish which mechanisms are of most general relevance to explaining the evolution of sexually selected traits.

METHODS SUMMARY

Study population. The Soay sheep of the St Kilda archipelago (57° 49′ N, 8° 34′ W) are a feral population of Neolithic domestic sheep[27] that have been studied on an individual basis since 1985.

Estimation of reproductive success. A total of 5,880 sheep sampled between 1980 and 2012 were genotyped at 51,135 single nucleotide polymorphisms (SNPs) on an Ovine SNP50 BeadChip[28]. Of the 38,404 polymorphic loci that passed quality control, 315 informative, unlinked SNPs from the chip were used to construct a pedigree to determine individual reproductive success.

***RXFP2* genotyping.** A total of 1,750 sheep (796 males and 954 females) sampled between 1990 and 2008 with information on annual reproductive success and annual survival up to 2011 were genotyped at a diagnostic SNP in strong linkage disequilibrium with the putative *RXFP2* alleles, Ho^{+} and Ho^{P} (ref. 6).

Detecting fitness differences between *RXFP2* genotypes. The relationship between *RXFP2* genotype and annual reproductive success, survival and overall fitness was modelled in males and females using a generalized linear mixed model framework with a Markov chain Monte Carlo (MCMC) method[29].

Determining selection coefficients at *RXFP2*. Mean values of survival, reproductive success and overall fitness for each *RXFP2* genotype were calculated from the raw data[3]. Relative fitness values were determined by dividing the mean value for all three genotypes by the maximum value (that is, the genotype with the highest fitness has a relative fitness equal to 1) and selection coefficients and equilibrium frequencies were determined using dominance or partial dominance models where appropriate[3].

Temporal trend in *RXFP2* allele frequencies. Gene-drop simulations ($n = 1{,}000$ iterations) were used to model the expected change in frequency of the allele Ho^{P} due to genetic drift only (that is, absence of directional selection) between 1990 and 2008 in pedigreed individuals[30].

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.E.J. (Susan.Johnston@ed.ac.uk) or J.S. (j.slate@shef.ac.uk).

METHODS

Study population. The Soay sheep of the St Kilda archipelago (57° 49′ N, 8° 34′ W) are a feral population of Neolithic domestic sheep[27] that have been studied on an individual basis since 1985. Animals were handled in strict accordance with UK Home Office ethical regulations and all work was licensed under the UK Animals (Scientific Procedures) Act 1986.

Pedigree construction. To determine individual reproductive success measures, a pedigree was constructed using molecular data for both maternity and paternity. Sheep with available blood and/or tissue samples ($n = 5{,}880$) were typed at 51,135 SNPs on the Ovine SNP50 BeadChip[28] using an Illumina Bead Array genotyping platform. Highly informative and unlinked SNPs were selected for parentage analysis by carrying out linkage-disequilibrium-based SNP pruning in the software PLINK v1.07 (ref. 31), with the following parameters: SNPs with minor allele frequency >0.4 retained, variance inflation factor = 1.01 and sliding windows of 50 SNPs with the window shifted 5 SNPs at each step. 315 SNP loci with a pairwise R^2 of <0.01 passed these criteria and were used for pedigree construction. Maternity and paternity of 5,626 sheep born between 1980 and 2012 were inferred simultaneously in the R package MasterBayes[32] implemented in R v2.14.0, with 20,000 iterations, a burn-in of 5,000 iterations and a thinning interval of 10 iterations. Sheep were included in the list of candidate parents if still alive during the rut preceding the spring the lamb was born in, and candidate parents were discarded if showing more than 8 mismatches with the progeny. Some males were not genotyped on the Ovine SNP50 BeadChip, but had previously been included in pedigrees constructed using 14-18 microsatellite loci[33]; these fathers were retained in the current pedigree when they had been assigned with >95% confidence, and there was no more than one mismatch between father and offspring or between the mother–father–offspring trio over all microsatellite loci.

Phenotypic data. Annual life history data were collected for Soay sheep during the period 1985 to 2011. Survival and reproductive success was recorded from November of a given year to the November of the following year for all individuals where this information was available; survival was scored as a binary trait and reproductive success was scored as the number of offspring surviving to at least the first November after birth (≥6 months old). Reproductive success was calculated for males only when they had at least one paternity or had been observed in the study area during the rut. A measure of annual 'overall fitness' was also calculated, where the contribution of an individual to the population count in the following year was calculated as the sum of individual survival and half of the number of offspring; this measure was transformed into integer values by multiplying all values by two. Models of fitness also included the covariate body weight (kg), which was measured during the August of the year the fitness measure was made. In total, there were 1,310 records on 796 males and 2,782 records on 954 females where all measures were available.

***RXFP2* genotyping.** A total of 1,750 sheep (796 males and 954 females) sampled between 1990 and 2008 with known phenotypic information were genotyped at a single diagnostic SNP in the 3′ untranslated region of *RXFP2* on ovine chromosome 10 (SNP ID: G100364-AS001072, developed by AgResearch Ltd, Invermay Agricultural Centre). The alleles at this diagnostic SNP are in strong linkage disequilibrium with putative alleles Ho^+ and Ho^P described in previous gene mapping papers; therefore, we refer to the diagnostic SNP genotypes in terms of their predicted genotype at *RXFP2* (ref. 6). We confirmed (1) the association of *RXFP2* with horn phenotype and (2) the suitability of use of this diagnostic SNP in this study: methods and results are presented in Supplementary Note 1. The diagnostic SNP was genotyped using a multiplex SNP-SCALE protocol[6,34], with additional animals typed on an Illumina BeadXpress Veracode GoldenGate SNP typing platform.

Detecting fitness differences between *RXFP2* genotypes using a mixed-model framework. The relationships between *RXFP2* genotype and annual reproductive success, annual survival and annual overall fitness were modelled using a generalized linear mixed model framework (GLMM) with an MCMC method in the R package MCMCglmm[29] in R v2.15.3. Models were fitted in males and females separately owing to differences in the distribution of fitness measures between the sexes. In males, reproductive success was modelled in all males and separately in lambs (age 1) and in adults (age 2+), as lamb males are much less likely to sire offspring. Survival was modelled with a binomial error structure, and reproductive success and overall fitness were modelled with Poisson error structures. Fixed effects included the age of measurement as a linear function, body weight and *RXFP2* genotype (fitted as a three level factor corresponding to genotypes Ho^+Ho^+, Ho^+Ho^P and Ho^PHo^P). Random effects were the animal identity (to account for repeated measures on individual sheep), year of birth and the year of fitness measurement, where the two latter effects accounted for variation attributed to specific environmental effects associated with these years. Effect sizes were estimated with posterior specification of previous distributions for random effect structures in the model. All models were run for N iterations and sampled 1,000 times after burn-in (see Supplementary Table 1 for specific values of N, burn-in and thinning interval for each model). Models were accepted if the independence of the samples in the posterior distribution (that is, the autocorrelation) was <0.1. Effect sizes and credible intervals for fixed and random effects were estimated from the posterior mode of the sampled iterations.

Determining selection coefficients at *RXFP2*. To determine selection coefficients and the expected frequency of *RXFP2* at equilibrium, it was necessary to calculate the relative fitness values of the genotypes, rather than their absolute values[3]. Therefore, we calculated the mean value of annual survival, annual reproductive success and annual overall fitness for each *RXFP2* genotype from the raw data in all sheep of both sexes ($n = 2{,}523$). Relative fitness values were determined by dividing the mean value for all three genotypes by the maximum value (that is, the genotype with the highest fitness will always have relative fitness equal to 1). In dominance or partial dominance models (that is, the two homozygotes are the most and least fit of the three genotypes), the relative fitnesses of the heterozygote and the less fit homozygote are denoted as $1 - hs$ and $1 - s$, respectively, where h and s are their respective selection coefficients. In cases where heterozygotes have the highest fitness (known as overdominance or heterozygote advantage), the relative fitnesses of each homozygote are denoted as $1 - s_1$ and $1 - s_2$, respectively, where s_1 and s_2 are the selection coefficients for their respective homozygotes. If overdominance exists, then an equilibrium frequency can be calculated[3] as $q = s_2/(s_1 + s_2)$. If the product of selection coefficients and effective population size (N_e) is much greater than 1, then selection is likely to be more important in determining changes in allele frequency than drift; N_e was previously estimated as 194 individuals using data from a subset of 190 Soay sheep typed on the Ovine SNP50 Beadchip[28]. The 95% confidence interval for each selection coefficient was estimated using a bootstrap method resampling the data 1,000 times, where the fitness values were calculated relative to a value of 1 for the heterozygote, and s_1 and s_2 calculated as for the overdominance model.

Temporal trend in *RXFP2* allele frequencies. Gene-drop simulations ($n = 1{,}000$ iterations) were used to model the expected change in frequency of the allele Ho^P due to genetic drift only (that is, absence of directional selection) between 1990 and 2008, in all pedigreed individuals during this period ($n = 4{,}738$), given an identical pedigree to the true one[30]. For each parent, a randomly chosen allele was transmitted to their offspring; individuals with one or two unknown parents were assigned the unknown allele according to the population allele frequencies in the year they were born. For each simulated pedigree, the change in allele frequency over the study period was modelled using linear regression in animals that had been genotyped at *RXFP2*, generating a distribution of regression slopes that are expected due to drift but no selection. The probability of obtaining the observed change in frequency due to drift alone was determined by comparing the slope of the linear regression in the observed data with the distribution of slopes from the gene-drop simulations. All simulations and regressions were performed in R v2.15.3.

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