Genetic architecture and lifetime dynamics of inbreeding depression in a wild mammal

Authors

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Abstract

Inbreeding depression is ubiquitous, but we still know little about its genetic architecture and precise effects in wild populations. Here, we combine long-term life-history data with 417K imputed SNP genotypes for 5,952 wild Soay sheep to explore inbreeding depression on a key fitness component, annual survival. Inbreeding manifests in long runs of homozygosity (ROH), which make up nearly half of the genome in the most inbred individuals. The ROH landscape varies widely across the genome, with islands where up to 87% and deserts where only 4% of individuals have ROH. The fitness consequences of inbreeding are severe; a 10% increase in individual inbreeding F_{ROH} is associated with a 60% reduction in the odds of survival in lambs, though inbreeding depression decreases with age. Finally, a genome-wide association scan on ROH shows that many loci with small effects and five loci with larger effects contribute to inbreeding depression in survival.

Introduction

Inbreeding depression, the reduced fitness of offspring from related parents, has been a core theme in evolutionary and conservation biology since Darwin [1]. The detrimental effects of inbreeding on a broad range of traits, individual fitness and population viability have now been recognized across the animal and plant kingdoms [1-9]. With the ongoing decline of animal populations [10] and global habitat fragmentation [11], rates of inbreeding are likely to accelerate, and so it is increasingly important to have a detailed understanding of its genetic causes and fitness consequences to inform conservation strategies. However, we still know very little about some of the most fundamental features of inbreeding depression in wild populations [7,12], such as its precise strength, how it varies across life-history stages [13-15], and if it is driven by loci with weak and/or strong deleterious effects on fitness. As genomic data proliferates for wild populations, it is increasingly possible to quantify the distribution of effect sizes at loci underpinning inbreeding depression. By determining this genetic architecture, we can improve our understanding of the relationship between inbreeding, purging and genetic rescue [7,16], with important implications for the persistence and conservation of small populations [17-19].

Inbreeding decreases fitness because it increases the fraction of the genome which is homozygous and identical-by-descent (IBD). This unmasks the effects of (partially-) recessive deleterious alleles or in rarer cases may decrease fitness at loci with heterozygote advantage [4,20]. While the probability of IBD at a genetic locus was traditionally estimated as the expected inbreeding coefficient based on a pedigree [21,22], modern genomic approaches enable us to gain a much more detailed picture. Genome-wide markers or whole genome sequences are now helping to unravel the genomic mosaic of homo- and heterozygosity and, unlike pedigree-based approaches, capture individual variation in homozygosity due to the stochastic effects of Mendelian segregation and recombination [12,23]. This makes it possible to quantify realized rather than expected individual inbreeding, and to measure IBD precisely along the genome [24,25].

An intuitive and powerful way of measuring IBD is through runs of homozygosity (ROH), which are long stretches of homozygous genotypes [26]. An ROH arises when two IBD haplotypes come together in an individual, which happens more frequently with increasing parental relatedness. The frequency and length of ROH in a population vary along the genome due to factors such as recombination, gene density, genetic drift and linkage disequilibrium[27-31] and extreme regions can potentially pinpoint loci under natural selection[24,31]. Regions with high ROH density, known as "ROH islands"[32], have low genetic diversity and high homozygosity and have been linked to loci under positive

selection in humans [31]. Regions where ROH are rare in the population, known as "ROH deserts", could be due to loci under balancing selection or loci harbouring strongly deleterious mutations under purifying selection [12,24,33–35]. Moreover, the abundance of ROH also varies among ROH length classes, which are shaped by the effective population size (N_e)[30,31,36,37] and suggested to vary in their deleterious allele load [38–40]. Longer ROH are a consequence of closer inbreeding and their relative abundance is indicative of recent N_e. This is because their underlying IBD haplotypes have a most recent common ancestor (MRCA) in the recent past with fewer generations for recombination to break them up. In contrast, shorter ROH are derived from more distant ancestors and their relative abundance in the population reflects N_e further back in time [41].

Individual inbreeding can be measured as the proportion of the autosomal genome in ROH (F_{ROH}), which is an estimate of realized individual inbreeding F [42]. F_{ROH} has helped to uncover inbreeding depression in a wide range of traits in humans and farm animals [5,29,43] and is often preferable to other SNP-based inbreeding estimators in terms of precision and bias [5,44–46]. While F_{ROH} condenses the information about an individual's IBD into a single number, quantifying the genomic locations of ROH across individuals makes it possible to identify the loci contributing to inbreeding depression and estimate their effect sizes [12]. As mapping inbreeding depression in fitness and complex traits requires large samples in addition to dense genomic data [37], the genetic architecture of inbreeding depression has mostly been studied in humans and livestock [47,48]. However, individual fitness will be different under natural conditions and consequently there is a need to study inbreeding depression in wild populations to understand its genetic basis in an evolutionary and ecological context. To date, only a handful of studies have estimated inbreeding depression using genomic data in the wild [13,14,49–52]. While these studies show that inbreeding depression in wild populations is more prevalent and more severe than previously thought, all of them used genome-wide inbreeding coefficients and did not explore the underlying genetic basis of depression.

The Soay sheep of St. Kilda provide an exceptional opportunity for a detailed genomic study of inbreeding depression. The Soay sheep is a primitive breed which was brought to the Scottish St. Kilda archipelago around 4000 years ago[53], and has survived on the island of Soay ever since. Although the Soay sheep have been largely unmanaged on Soay, there is written and genomic evidence of an admixture event with the now extinct Dunface breed approximately 150 years or 32 generations ago [54]. In 1932, 107 Soay sheep were transferred to the neighbouring island of Hirta where they are unmanaged. On Hirta the population increased and nowadays fluctuates between 600 and 2200 individuals. A part of the population in Village Bay became the subject of a long-term

individual-based study, and detailed life-history, pedigree and genotype data have been collected for most individuals born since 1985[53].

Here, we combine annual survival data for 5,952 free-living Soay sheep over a 35 year period with 417K partially imputed genome-wide SNP markers to estimate the precise effects and genetic basis of inbreeding depression. First, we quantify the genomic consequences of inbreeding through patterns of ROH among individuals and across the genome. We then calculate individual genomic inbreeding coefficients F_{ROH} to model inbreeding depression in annual survival and estimate its strength and dynamics across the lifetime. Finally, we explore the genetic architecture of inbreeding depression using a mixed-model based genome-wide association scan on ROH to shed light on whether depression is caused by many loci with small effects, few loci with large effects or a mixture of both.

Results

Genotyping and imputation.

All study individuals have been genotyped on the Illumina Ovine SNP50 BeadChip assaying 51,135 SNPs. In addition, 189 individuals have been genotyped on the Ovine Infinium High-Density chip containing 606,066 SNPs. To increase the genomic resolution for our analyses, we combined autosomal genotypes from both SNP chips with pedigree information to impute missing SNPs in individuals genotyped at lower marker density using AlphaImpute [55]. Cross-validation showed that imputation was successful, with a median of 99.3% correctly imputed genotypes per individual (Supplementary Table 1). Moreover, the inferred inbreeding coefficients F_{ROH} were very similar when comparing individuals genotyped on the high-density chip (median F_{ROH} = 0.239) and individuals with imputed SNPs (median F_{ROH} = 0.241), indicating no obvious bias in the abundance of inferred ROH based on imputed data (Supplementary Figure 1). After quality control, the genomic dataset contained 417,373 polymorphic and autosomal SNPs with a mean minor allele frequency (MAF) of 23% (Supplementary Figure 2) and a mean call rate of 99.5% across individuals.

Patterns of inbreeding in the genome.

We first explored how inbreeding and a long-term small population size (estimated N_e = 194, [56]) shaped patterns of ROH in Soay sheep (Figure 1). Individuals had a mean of 194 ROH (sd = 11.6) longer than 1.2 Mb, which on average made up 24% of the autosomal genome (i.e. mean F_{ROH} = 0.24, range = 0.18-0.50, Supplementary Figure 3). The mean individual inbreeding coefficient F_{ROH} of sheep born in a given year remained constant over the course of the study period (Supplementary

Figure 4). Among individual variation in ROH length was high: The average ROH in the seven most inbred sheep was more than twice as long as ROH in the seven least inbred sheep (6.83 Mb vs. 2.72 Mb, respectively; Figure 1A) although the average number of ROH was similar (170 vs. 169, respectively).

The abundance of ROH in Soay sheep also varied considerably among ROH length classes (Figure 1B). The largest fraction of IBD in the population were ROH between 2.4 and 4.9 Mb originating around 8 to 16 generations ago, which made up 8.1 % of an individual's genome on average. Long ROH > 19.5 Mb were found in 38.2% of individuals (Figure 1B). However, long ROH made up on average only 0.6 % of the genome of the least inbred individuals with pedigree inbreeding F_{ped} < 0.1. In contrast, long ROH extended over 7% and 18% of the genome in inbred individuals with F_{ped} > 0.1 and F_{ped} > 0.2, respectively (Supplementary Figure 5).

The frequency of ROH in the population varied widely across the genome (Figure 1C). We scanned ROH in non-overlapping 500Kb windows, and classified the 0.5% windows with the highest ROH density as ROH islands and the 0.5% windows with the lowest ROH density as ROH deserts [31]. The top ROH island on chromosome 1 (227-227.5Mb) contained ROH in 87% of individuals, while only 4.4% of individuals had an ROH in the top ROH desert on chromosome 11 (58.5-59Mb, see Supplementary Table 2 for a list of the top ROH deserts and islands).

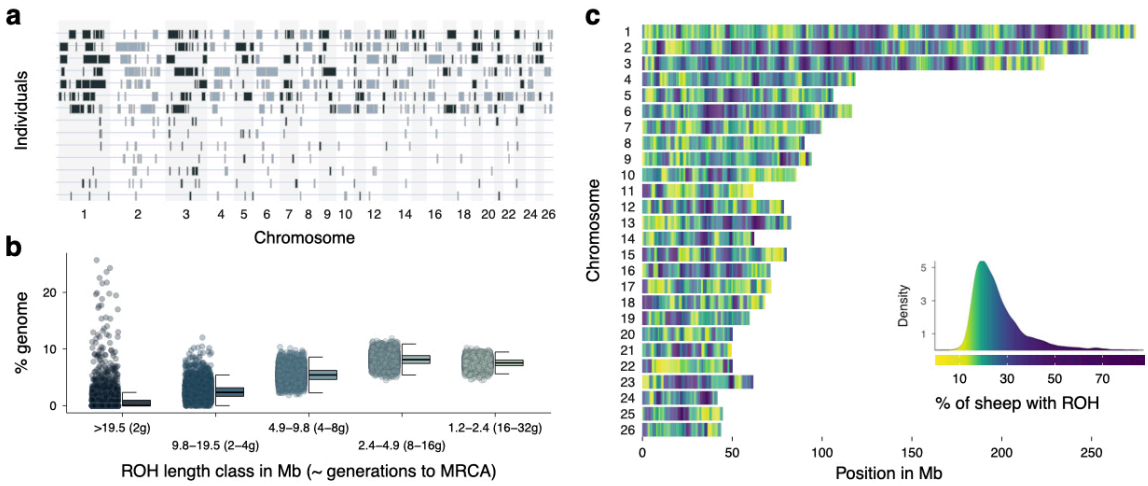

Figure 1: Runs of homozygosity (ROH) variation among individuals and across the genome. a ROH longer than 5 Mb in the seven individuals with the highest inbreeding coefficients F_{ROH} in the seven top rows and the seven individuals with the lowest F_{ROH} in the seven bottom rows. **b** Distribution of ROH among different length classes. Each data point represents the proportion of ROH of a certain length class within an individual's autosomal genome. ROH length classes were categorized by their expected average physical length when the underlying haplotypes had a most recent common ancestor (MRCA) 2 to 32 generations (g) ago. **c** Genome-

156 wide ROH density among all 5,952 individuals in non-overlapping 500 Kb windows. The colour gradient has
157 been scaled according to the ROH density, which is shown in the figure legend.

158 **ROH density and recombination rate**.

159 The wide variation in ROH density along the genome could be partially explained by recombination,
160 because regions with high recombination rate produce shorter ROH, and these are less likely to be
161 detected by ROH calling algorithms[30]. Notably, recombination by itself does not impact the
162 underlying true proportion of IBD, but only affects the ROH length distribution. Consequently,
163 regions with high recombination could create putative ROH deserts without a change in the true
164 levels of IBD, because short ROH are less likely to be called [30]. To evaluate how much variation in
165 ROH density along the genome is due to recombination rate variation and how much of it is tracking
166 the underlying levels of IBD, we constructed a linear mixed model with ROH density (proportion of
167 individuals with ROH) measured in 500Kb windows as response variable, window recombination
168 rate in cM/Mb based on the Soay sheep linkage map [57] and window SNP heterozygosity as fixed
169 effects as well as a chromosome identifier as random effect.

170

171 Recombination rate and heterozygosity together explained 42% of the variation in ROH density
172 (marginal R^2 = 0.42, 95% CI [0.40, 0.44], Supplementary Table 3A), with the majority of variation
173 explained by heterozygosity (semi-partial R^2 = 0.38, 95% CI [0.36, 0.40], Figure 2B) and only around
174 4% explained by recombination rate (semi-partial R^2 = 0.04, 95% CI [0.02, 0.07], Figure 2A and
175 Supplementary Figure 6 for a chromosome-wise plot). The pattern is similar when re-running the
176 model only on windows identified as ROH islands and deserts, where ROH density is largely
177 explained by heterozygosity (semi-partial R^2 = 0.89, 95% CI [0.83, 0.94], Figure 2D), with only a small
178 proportion of the variation explained by recombination rate variation (semi-partial R^2 = 0.07, 95% CI
179 [0.01, 0.12], Figure 2C). Consequently, although recombination rate impacts ROH lengths and hence
180 ROH detection probabilities, this accounts for only a small proportion of the variation in detected
181 ROH density, which mostly reflects the underlying patterns of IBD along the genome.

182

183 Lastly, we explored how much variation in ROH density was explained by recombination when using
184 different minimum ROH thresholds. We repeated the analysis with a dataset based on a minimum
185 ROH length threshold of 0.4Mb, and a second dataset with a minimum ROH length of 3Mb
186 (Supplementary Table 3B,C). Compared to the original dataset with a minimum ROH length of
187 1.2Mb, recombination explained less variation in ROH density in the dataset including shorter ROH
188 (semi-partial R^2 = 0.01, 95% CI [0.00, 0.04]) and more variation in the dataset consisting only of longer

ROH (semi-partial R^2 = 0.08, 95% CI [0.06, 0.11]). Consequently, recombination rate variation has a larger impact on the detected abundance of longer ROH across the genome.

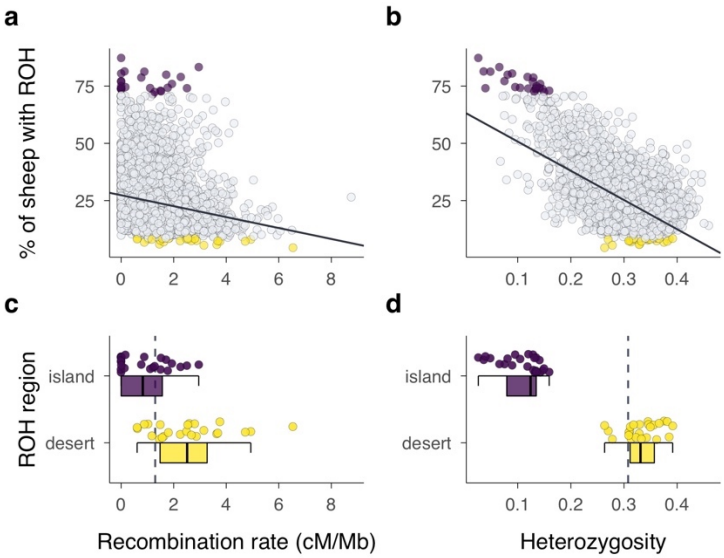

Figure 2: Correlates of ROH density variation across the genome. Runs of homozygosity (ROH) density, recombination rate and heterozygosity were quantified in non-overlapping 500 Kb windows, with each point representing one window. The top 0.5% of windows with the highest and lowest ROH density in the population, termed ROH islands (n=24) and deserts (n=24), are coloured in purple and yellow respectively in all four plots. **a** Relationship of ROH density and recombination rate. **b** Relationship of ROH density and SNP heterozygosity. **c** Recombination rate within ROH islands and deserts. **d** Heterozygosity within ROH islands and deserts. Solid lines in a and b are linear regression lines and dashed lines in c and d are genome-wide means. Boxplots show the median as centre line with the bounds of the box as 25th and 75th percentiles and upper and lower whiskers as largest and smallest value but no further than 1.5 * inter-quartile range from the hinge. Source data for this figure are also provided as source data file.

Inbreeding depression in survival.

Survival is a key fitness component. In Soay sheep, more than half of all individuals die over their first winter, minimizing their chances to reproduce (Supplementary Figure 7). Sheep survival is assessed through routine mortality checks which are conducted throughout the year. Over 80% of sheep in the study area are found after their death [49], resulting in a total of 15889 annual survival observations for 5952 sheep. The distribution of individual inbreeding coefficients F_{ROH} in different age classes revealed that highly inbred individuals rarely survive their early years of life and never reach old ages (Figure 3A). However, the strength of inbreeding depression appeared to decline at older ages (Figure 3B). For example, in sheep older than four years, the proportion of survivors among the most inbred individuals was only marginally lower than among the least inbred individuals (Figure 3B).

We modelled the strength of inbreeding depression across the lifetime using an animal model with a binomial error distribution and annual survival as a response variable. Overall, the effect of inbreeding on survival was strong: in lambs (age 0), a 10% increase in F_{ROH} was associated with a 0.4 multiplicative change in the odds of survival (Odds-ratio, OR [95% credible interval, CI] = 0.40 [0.30, 0.53], Supplementary Table 4), or a 60% reduction (1 - 0.40) in the odds of survival. This translates into non-linear survival differences on the probability scale. For example, a male non-twin Soay sheep lamb with an F_{ROH} 10% above the mean had a 23% lower probability of surviving its first winter compared to an average lamb (F_{ROH} = 0.34 vs F_{ROH} = 0.24; Figure 3C). Across the lifetime, the model estimates for the interactions between F_{ROH} and the different life stages predicted a decrease in the strength of inbreeding depression in later life stages (Figure 3C) with the largest predicted difference between early (age 1,2) and late life (age 5+, OR [95% CI] = 2.03 [1.08, 3.82], Supplementary Table 4).

We next estimated the inbreeding load in Soay sheep as the diploid number of lethal equivalents 2B. Lethal equivalents are a concept rooted in population genetics, where one lethal equivalent is equal to a group of mutations which, if dispersed across individuals, would cause one death on average [58]. We followed suggestions by Nietlisbach et al [59] and refitted the survival model with a Poisson distribution and logarithmic link function using a simplified model structure without interactions for a better comparability across studies. This gave an estimate of *2B* = 4.57 (95% CI 2.61-6.55) lethal equivalents for Soay sheep annual survival.

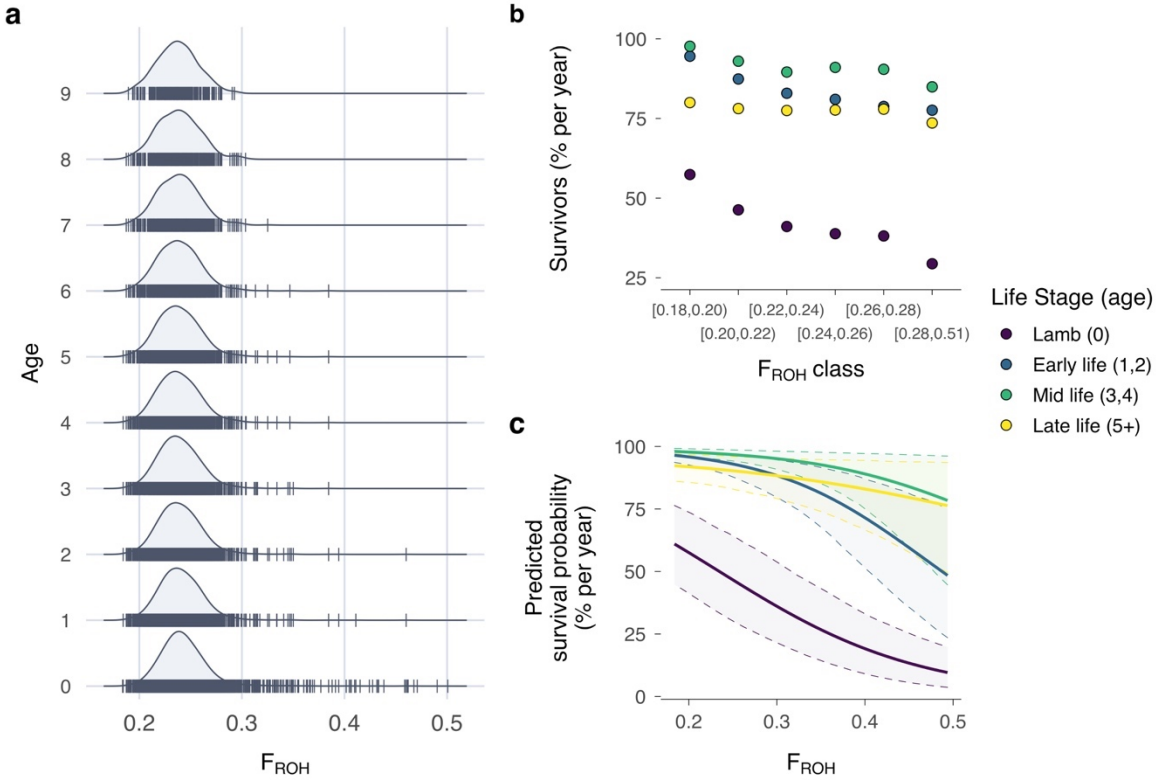

Figure 3: Inbreeding depression in annual survival. a Distributions of inbreeding coefficients F_{ROH} in Soay sheep age classes ranging from 0 to 9 years. **b** Proportion of surviving individuals per year in four different life stages and among different F_{ROH} classes. As highly inbred individuals are relatively rare, the last class spans a wider range of inbreeding coefficients. Source data for this figure are also provided as source data file. **c** Predicted survival probability and 95% credible intervals over the range of inbreeding coefficients F_{ROH} for each life stage, while holding sex and twin constant at 1 (male) and 0 (no twin). The predictions for the later life stages classes exceed the range of the data but are shown across the full range for comparability.

Genetic architecture of inbreeding depression.

To quantify the survival consequences of being IBD at each SNP location, we used a modified genome-wide association study (GWAS). Unlike in traditional GWAS where p-values of additive SNP effects are of interest, we analysed the effects of ROH status for both alleles at every SNP. Specifically, at a diallelic locus, ROH result either from two IBD haplotypes containing allele A or from two IBD haplotypes containing allele B. If strongly deleterious recessive alleles exist in the population, they could be associated with ROH based on allele A haplotypes or ROH based on allele B haplotypes. To test this, we constructed a binomial mixed model of annual survival for each SNP position. In each model, we fitted two ROH status predictors. The first predictor was assigned a 1 if allele A was homozygous and part of an ROH and a 0 otherwise. The second predictor was assigned a 1 if allele B was homozygous and part of an ROH and a 0 otherwise. Model estimates and p-values for these

258 two predictors therefore reflect whether ROH are associated with survival consequences at each SNP
259 location and for each allele. In the GWAS model, we also controlled for the additive SNP effect and
260 mean individual inbreeding F_{ROH} (based on all autosomes except for the focal chromosome),
261 alongside a range of other individual traits and environmental effects (see Methods for details).
262
263 A GWAS on allele-wise ROH status can detect deleterious recessive alleles at specific regions when
264 ROH effects reach genome-wide significance. Moreover, the distribution of ROH status effects across
265 the genome can also be informative of the overall number of deleterious recessive alleles
266 contributing to inbreeding depression through ROH. Under the null hypothesis that ROH status does
267 not have an effect on survival at any SNP position, we would expect a 50/50 distribution of negative
268 and positive ROH status estimates due to chance. In contrast, we found many more negative than
269 positive effects of ROH status on survival across the genome than expected by chance (Figure 4A,
270 4B; 465K neg. vs. 354K pos.; exact binomial test $p = 2.2 * 10^{-16}$). Moreover, the proportion of negative
271 ROH effects increases for larger model estimates (Figure 4A) and smaller p-values (Figure 4B). We
272 tested this statistically using two binomial generalized linear models (GLMs), with effect direction as
273 binary response, and model estimate and p-value, respectively, as predictors. ROH effects were
274 more likely to be negative when their model estimate was larger (log-OR [95% CI] = 0.35 [0.344,
275 0.358]) and when their p-value was smaller (log-OR [95% CI] = -3.82 [-3.84, -3.80]). Consequently, it
276 is likely that a large number of recessive deleterious alleles contribute to inbreeding depression,
277 which manifest in negative ROH effects spread across many loci.
278
279 The GWAS revealed genome-wide significant ROH effects in seven regions on chromosomes 3 (two
280 regions), 10, 14, 18, 19 and 23 (Figure 4C, Supplementary Table 5). In five of these regions, ROH
281 status for one of the alleles was associated with negative effects on survival, likely caused by relatively
282 strongly deleterious recessive alleles. ROH in two further regions on chromosomes 3 and 19 were
283 associated with increased survival probabilities, possibly due to haplotypes with positive effects on
284 survival. To explore the genomic regions with large ROH effects further, we quantified the ROH
285 density and SNP heterozygosity in 2Mb windows around the top GWAS hits (Supplementary Figure
286 8). Strongly deleterious recessive alleles might be expected to occur in regions of elevated
287 heterozygosity where they are rarely expressed in their homozygous state. Heterozygosity was
288 higher than average around the top SNPs on chromosomes 10, 14, 18 and 23, and ROH frequency
289 was lower around the top SNPs on chromosomes 10, 18, 19 and 23, but overall, but we did not
290 observe a convincing pattern of genetic diversity across the five regions harbouring strongly
291 deleterious mutations (Supplementary Figure 8).

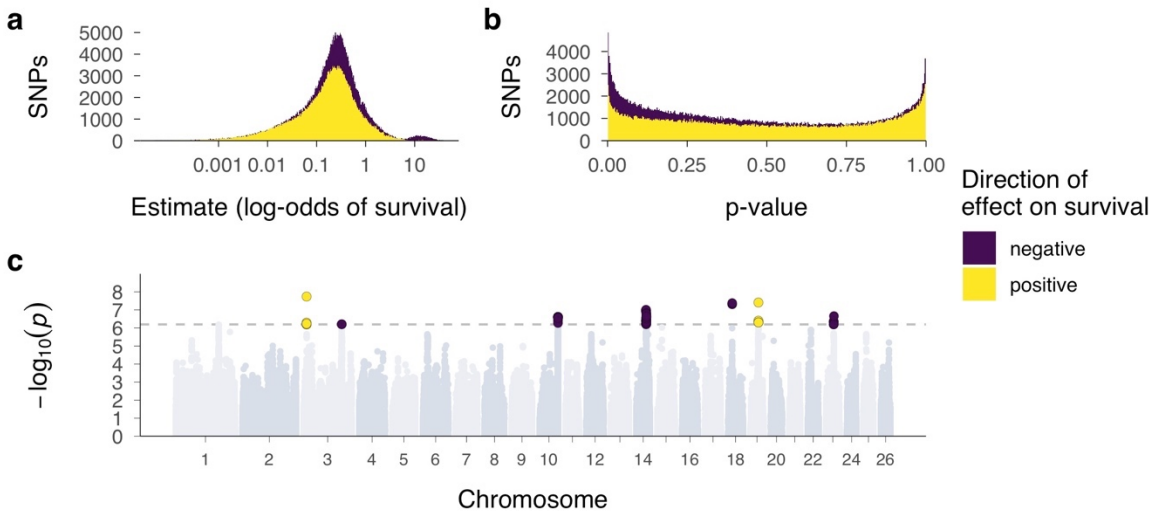

Figure 4: GWAS of SNP-wise ROH status effects on annual survival. Regional inbreeding depression was conceptualised and tested using two binary ROH status predictors. One of the predictors quantified the ROH status of allele A (in ROH = 1, not in ROH = 0), while the other quantified the ROH status of allele B. **a** Distribution of effect sizes for SNP-wise ROH status effects. **b** Distribution of p-values for SNP-wise ROH status effects. The yellow histograms showing positive effects are superimposed on top of the purple histograms showing negative effects to highlight a substantially larger proportion of negative ROH status effects than expected by chance. **c** Manhattan plot of the ROH status p-values across the genome. The dotted line marks the genome-wide significance threshold for a Bonferroni correction which was based on the effective number of tests when accounting for linkage disequilibrium.

Discussion

The Soay sheep on St. Kilda have existed at a small population size in relative isolation for thousands of years [53]. As a consequence, levels of IBD are high in the population and ROH make up nearly a quarter of the average autosomal Soay sheep genome. Although this is still an underestimate as we only analysed ROH longer than 1.2Mb, it is three times as high as the average F_{ROH} estimated across 78 mammal species based on genome-sequence data [60] and only slightly lower than in some extremely inbred and very small populations such as mountain gorillas [61], Scandinavian grey wolves [24] or Isle Royale wolves [17].

The distribution of ROH length classes can provide insights into population history and levels of inbreeding [24,31,36]. In Soay sheep, the largest fraction of IBD was comprised of ROH with lengths between 1.2 and 4.9 Mb. These ROH originate from haplotypes around 8-32 generations ago and their relative abundance reflects a smaller N_e of the population in the recent past. While there is

318 considerably higher uncertainty in estimating the time to the MRCA for ROH when these are
319 measured based on physical rather than genetic map lengths [12], this corroborates with historical
320 knowledge: The Soay sheep population was indeed smaller in the early 20th century when 107 sheep
321 were translocated from the island of Soay to their current location on Hirta, after the last humans left
322 St. Kilda [53]. Current levels of inbreeding were most visible in the variation in long ROH (> 19.5Mb)
323 which made up less than 1% of the genome of the least inbred individuals on average, but 18% of
324 the genome of highly inbred individuals with pedigree inbreeding coefficients F_{ped} > 0.2
325 (Supplementary Figure 5).
326
327 The abundance of ROH in the population also varied substantially across the genome. In the most
328 extreme ROH deserts and islands, only 4% of individuals and up to 87% of individuals had ROH,
329 respectively, compared to 24% on average. These detected ROH islands and deserts could be
330 regions with genuinely low or high levels of IBD due to genetic drift or natural selection, but they
331 might also be a consequence of low or high recombination rates [31]. However, recombination itself
332 cannot change the true abundance of IBD[30]. Instead, ROH with a given coalescent time will be
333 shorter in regions with high recombination and are less likely to be detected by ROH calling
334 algorithms[30]. We modelled this and found that only 4% of the variation in ROH density across the
335 genome was explained by variation in recombination rate, but the impact of recombination was
336 greater on the detected densities of longer ROH. Consequently, the association between ROH
337 density and recombination could change with both the minimum ROH threshold and the average
338 inbreeding levels in a population. In line with the low genome-wide effects of recombination on ROH
339 density, many of the ROH islands and deserts also had very similar recombination rates (Figure 2C).
340 Ruling out recombination as a major driver for ROH islands and deserts opens up the possibility for
341 future studies to compare the extreme ROH density in islands and deserts to expectations under
342 simulated neutral scenarios to test for positive and purifying selection, respectively [24,31].
343
344 ROH deserts might for example harbour loci contributing to inbreeding depression, as strongly
345 deleterious alleles are likely to cause ROH to be rare in their genomic vicinity due to purifying
346 selection removing homozygous haplotypes [7,24,31]. However, because of the near absence of ROH,
347 genome wide association analyses are unlikely to pick up deleterious effects due to a lack of
348 statistical power, and indeed none of our top GWAS hits was located in a ROH desert. An alternative
349 option is to test for deficits of homozygous genotypes under expected frequencies, as deployed in
350 farm animals and plants to identify embryonic lethals [33-35], though these methods require either an
351 experimental setup or very large sample sizes with tens to hundreds of thousands of individuals. In

352 contrast, ROH islands with very high ROH abundances probably contain very few recessive
353 deleterious alleles, as these are regularly exposed to selection when homozygous and hence likely
354 to be purged from the population. Instead, it is possible that ROH islands have emerged around loci
355 under positive selection [29,31] through hard selective sweeps [30].
356
357 We have only recently begun to understand the precise consequences of inbreeding for individual
358 fitness in natural populations. In Soay sheep, we found that the odds of survival decreased by 60%
359 with a mere 10% increase in F_{ROH}, adding to a small yet growing body of genomic studies reporting
360 stronger effects of inbreeding depression in wild populations than assumed in pre-genomics times
361 [13,14,49,50,52]. Other recent examples include lifetime breeding success in red deer, which is reduced
362 by up to 95% in male offspring from half-sib matings [13] and lifetime reproductive success in helmeted
363 honeyeaters, which is up to 90% lower with a 9% increase in homozygosity [50]. The traditional way to
364 compare inbreeding depression among studies is to estimate the inbreeding load of a population
365 using lethal equivalents [58], although differences in methodology and inbreeding estimates can make
366 such direct comparisons difficult [59]. We estimated the diploid number of lethal equivalents 2B for
367 Soay sheep annual survival at 4.57 (95% CI 2.61-6.55). While this is a low to moderate inbreeding
368 load compared to the few available estimates from wild mammals obtained from appropriate
369 statistical models [59], none of these estimates are based on genomic data and they vary in their exact
370 fitness measure as well as the degree to which they control for environmental and life-history
371 variation. As such, average estimates of lethal equivalents might change in magnitude with the
372 increasing use of genomics in individual-based long-term studies.
373
374 Inbreeding depression is dynamic across life, and genomic measures are starting to unravel how
375 inbreeding depression affects fitness at different life-stages in wild populations [13,14,49]. Under the
376 mutation accumulation hypothesis [62], the adverse effects of deleterious mutations expressed late in
377 life should become stronger as selection becomes less efficient. Assuming mutation accumulation,
378 inbreeding depression is expected to increase with age too [63], but empirical evidence is sparse
379 [15,64,65]. In contrast, we showed that inbreeding depression in Soay sheep becomes weaker at later
380 life stages. In addition, the sample for each successive age class consists of increasingly outbred
381 individuals (Figure 3A) due to a higher death rate among inbred individuals earlier in life. This
382 suggests that the effects of intragenerational purging [66] outweigh mutation accumulation in shaping
383 the dynamics of inbreeding depression across the lifetime.
384

385 The effect size distribution of loci underpinning inbreeding depression has to our knowledge not
386 been studied in wild populations using fitness data, although deleterious mutations have been
387 predicted from sequencing data, for example in ibex and Isle Royale wolves [17,18]. Theoretical
388 predictions about the relative importance of weakly and strongly deleterious (partially-) recessive
389 alleles will depend on many factors, such as the distribution of dominance and selection coefficients
390 for mutations relative to the effective population size, and the frequency of inbreeding [67,68]. However,
391 we could expect that small populations purge largely deleterious recessive mutations more
392 efficiently as these are more frequently exposed to selection in the homozygous state [7,16,19], while
393 weakly deleterious mutations can more often drift to higher frequencies. We estimated the effect of
394 ROH status on Soay sheep survival for each of the two alleles at every SNP position within a GWAS
395 framework. The effect size distribution revealed predominantly negative effects of ROH status on
396 survival, particularly towards larger model estimates, showing that many alleles with weakly
397 deleterious effects (or at low frequencies) probably contribute to inbreeding depression in survival.
398
399 Associations between ROH and survival reached genome-wide significance in seven regions on six
400 chromosomes. In two of these regions, allele-specific ROH are predicted to increase survival, a
401 fascinating observation we intend to explore in more detail but which is beyond the scope of this
402 manuscript. In five further regions, ROH caused significant depression in survival, presumably due
403 to loci harbouring strongly deleterious recessive alleles. This is unexpected, as Soay sheep have a
404 long-term small population size with an estimated N_e of 197 [56], and strongly deleterious mutations
405 should be rapidly purged. On the one hand, it is possible that genetic drift counteracted the effects
406 of purifying selection and has allowed deleterious mutations to increase in frequency and be
407 detected in a GWAS. On the other hand, a relatively recent admixture event with the Dunface sheep
408 breed around 150 years ago [54] could have introduced deleterious variants into the population and
409 recent selection has not been efficient enough to purge them from the population yet. Identifying
410 the loci harbouring these strongly deleterious alleles will be challenging as ROH overlapping a given
411 SNP vary in length among individuals, which makes it difficult to pinpoint an exact effect location.
412 Nevertheless, we have shown that it is possible to identify the haplotypes carrying deleterious alleles
413 with large effects. The frequencies of such haplotypes could be monitored in natural populations,
414 and individuals carrying them could be selected against in conservation breeding programs. To sum
415 up, our study shows how genome-wide marker information for a large sample of individuals with
416 known fitness can deepen our understanding of the genetic architecture and lifetime dynamics of
417 inbreeding depression in the wild
418

Methods

Study population, pedigree assembly and survival measurements.

The Soay sheep (*Ovis aries*) is a primitive sheep breed descended from Bronze Age domestic sheep and has lived unmanaged on island of Soay in St. Kilda archipelago, Scotland for thousands of years. When the last human inhabitants left St. Kilda in 1932, 107 Soays were transferred to the largest island, Hirta, and have roamed the island freely and unmanaged ever since. The population increased and fluctuates nowadays between 600 and 2200 individuals. A part of the population in the Village Bay area of Hirta (57 49'N, 8 34'W) has been the subject of a long-term individual based study since 1985 [53]. Most individuals born in the study area (95%) are ear-tagged and DNA samples are obtained from ear punches or blood sampling. Routine mortality checks are conducted throughout the year with peak mortality occurring at the end of winter and beginning of spring. Overall, around 80% of deceased animals are found [49]. For the analyses in this paper, survival was defined as dying (0) or surviving (1) from the 1st May of the previous year to the 30th April of that year, with measures available for 5952 individuals from 1979 to 2018. Annual survival data was complete for all individuals in the analysis, as the birth year was known and the death year of an individual was known when it has been found dead during one of the regular mortality checks on the island. We focused on annual measures as this allowed us to incorporate the effects of age and environmental variation.

To assemble the pedigree, we inferred parentage for each individual using 438 unlinked SNP markers from the Ovine SNP50 BeadChip, on which most individuals since 1990 have been genotyped [69]. Based on these 438 markers, we inferred pedigree relationships using the R package Sequoia [70]. In the few cases where no SNP genotypes were available, we used either field observations (for mothers) or microsatellites [71]. All animal work was carried out in compliance with all relevant ethical regulations for animal testing and research according to UK Home Office procedures and was licensed under the UK Animals (Scientific Procedures) Act of 1986 (Project License no. PPL70/8818).

Genotyping.

We genotyped a total of 7,700 Soay on the Illumina Ovine SNP50 BeadChip containing 51,135 SNP markers. To control for marker quality, we first filtered for SNPs with minor allele frequency (MAF) > 0.001, SNP locus genotyping success > 0.99 and individual sheep genotyping success > 0.95. We then used the check.marker function in GenABEL version 1.8-0 [72] with the same thresholds, including identity by state with another individual < 0.9. This resulted in a dataset containing 39,368

453 polymorphic SNPs in 7700 sheep. In addition, we genotyped 189 sheep on the Ovine Infinium HD
454 SNP BeadChip containing 606,066 SNP loci. These sheep were specifically selected to maximise the
455 genetic diversity represented in the full population as described in Johnston et al. [57]. As quality
456 control, monomorphic SNPs were discarded, and SNPs with SNP locus genotyping success > 0.99
457 and individual sheep with genotyping success > 0.95 were retained. This resulted in 430,702
458 polymorphic SNPs for 188 individuals. All genotype positions were based on the Oar_v3.1 sheep
459 genome assembly (GenBank assembly ID GCA_000298735.1 [73])
460
461 **Genotype imputation.**
462 In order to impute genotypes to high density, we merged the datasets from the 50K SNP chip and
463 from the HD SNP chip using PLINK v1.90b6.12 with --bmerge [74]. This resulted in a dataset with
464 436,117 SNPs including 33,068 SNPs genotyped on both SNP chips. For genotype imputation, we
465 discarded SNPs on the X chromosome and focused on the 419,281 SNPs located on autosomes. The
466 merged dataset contained nearly complete genotype information for 188 individuals which have
467 been genotyped on the HD chip, and genotypes at 38,130 SNPs for 7700 individuals which have
468 been genotyped on the 50K chip. To impute the missing SNPs, we used AlphaImpute v1.98 [55], which
469 combines information on shared haplotypes and pedigree relationships for phasing and genotype
470 imputation. AlphaImpute works on a per-chromosome basis, and phasing and imputation are
471 controlled using a parameter file (for the exact parameter file, see analysis code). Briefly, we phased
472 individuals using core lengths ranging from 1% to 5% of the SNPs on a given chromosome over 10
473 iterations, resulting in a haplotype library. Based on the haplotype library, missing alleles were
474 imputed using the heuristic method over five iterations which allowed us to use genotype
475 information imputed in previous iterations. We only retained imputed genotypes for which all
476 phased haplotypes matched and did not allow for errors. We also discarded SNPs with call rates
477 below 95% after imputation. Overall, this resulted in a dataset with 7691 individuals, 417,373 SNPs
478 and a mean genotyping rate per individual of 99.5 % (range 94.8%-100%).
479 To evaluate the accuracy of the imputation we used 10-fold leave-one-out cross-validation. In each
480 iteration, we masked the genotypes unique to the high-density chip for one random individual that
481 had been genotyped at high-density (HD) and imputed the masked genotypes. This allowed a direct
482 comparison between the true and imputed genotypes. The imputation accuracy of the HD
483 individuals should reflect of the average imputation accuracy across the whole population, because
484 HD individuals were selected to be representative of the genetic variation observed across the
485 pedigree (see Johnston et al., 2016 for details).
486

487 **ROH calling and individual inbreeding coefficients.**

488 The final dataset contained genotypes at 417,373 SNPs autosomal SNPs for 5925 individuals for
489 which annual survival data was available. We called runs of homozygosity (ROH) with a minimum
490 length of 1200Kb and spanning at least 50 SNPs with the --homozyg function in Plink [74] and the
491 following parameters: --homozyg-window-snp 50 --homozyg-snp 50 --homozyg-kb 1200 --homozyg-
492 gap 300 --homozyg-density 200 --homozyg-window-missing 2 --homozyg-het 2 --homozyg-window-
493 het 2. We chose 1200Kb as the minimum ROH length because between-individual variability in ROH
494 abundance becomes very low for shorter ROH. Moreover, ROH of length 1200Kb extend well above
495 the LD half decay in the population, thus capturing variation in IBD due to more recent inbreeding
496 rather than linkage disequilibrium (Supplementary Figure 9). The minimum ROH length of 1200Kb
497 also reflects the expected length when the underlying IBD haplotypes had a most recent common
498 ancestor haplotype 32 generations ago, calculated as (100 / (2*g)) cM / 1.28 cM/Mb where g is 32
499 generations and 1.28 is the sex-averaged genome-wide recombination rate in Soay sheep [41,57]. To
500 plot the ROH length distribution, we used the same formula to cluster ROH according to their
501 physical length into length classes with expected MRCA ranging from 2-32 generations ago (Figure
502 1B). Notably, ROH with the same physical length can have different coalescent times in different
503 parts of the genome, causing a higher variance around the expected mean length than ROH
504 measured in terms of genetic map length [12,41]. We then calculated individual inbreeding coefficients
505 F_{ROH} by summing up the total length of ROH for each individual and dividing this by the total
506 autosomal genome length [42] (2,452Mb).

507

508 **ROH landscape and recombination rate variation.**

509 To quantify variation in population-wide ROH density and its relationship with recombination rate
510 and SNP heterozygosity across the genome, we used a sliding window approach. For all analyses,
511 we calculated these estimates in 500Kb non-overlapping sliding windows comparable to similar
512 studies [24,30] ; each window contained 85 SNPs on average. Specifically, we first calculated the
513 number of ROH overlapping each SNP position in the population using PLINK --homozyg. We then
514 calculated the mean number of ROH overlapping SNPs in 500 Kb non-overlapping sliding windows
515 in the population (Figure 1C). To estimate the top 0.5% ROH deserts and islands [31], windows with
516 less than 35 SNPs (the percentile of windows with the lowest SNP density) were discarded. To
517 estimate the impact of recombination rate on ROH frequency across the genome, we then quantified
518 the recombination rate in 500Kb windows using genetic distances from the Soay sheep linkage map
519 [75]. Window heterozygosity was calculated as the mean SNP heterozygosity of all SNPs in a given
520 window. Next, we constructed a linear mixed model in lme4 [76] with population-wide ROH density

521 (defined as the proportion of individuals with ROH) per window as response, window recombination
522 rate and heterozygosity as fixed effects and chromosome ID as random intercept (Supplementary
523 Table 3). The fixed effects in the model were standardised using z-transformation. We estimated the
524 relative contribution of recombination and heterozygosity to variation in ROH density by
525 decomposing the marginal R^2 of the model [77] into the variation explained uniquely by each of the
526 two predictors using semi-partial R^2 as implemented in the partR2 package [78], with 95% confidence
527 intervals obtained by parametric bootstrapping.
528
529 **Modelling inbreeding depression in survival.**
530 We modelled the effects of inbreeding depression in annual survival using a Bayesian animal model
531 in INLA [79]. Annual survival data consists of a series of 1s followed by a 0 in the year of a sheep's death,
532 or only a 0 if it died as a lamb, and we consequently modelled the data with a binomial error
533 distribution and logit link. We used the following model structure:
534

$$\begin{aligned}\Pr(\mathrm{surv}_i = 1) = \mathrm{logit}^{-1}(\beta_0 + \mathrm{F}_{\mathrm{ROH_i}}\beta_1 + \mathrm{earlyLife}_i\beta_2 + \mathrm{midLife}_i\beta_3 + \mathrm{lateLife}_i\beta_4 + \mathrm{sex}_i\beta_5 + \\ \mathrm{twin}_i\beta_6 + \mathrm{F}_{\mathrm{ROH}_i}\mathrm{earlyLife}_i\beta_7 + \mathrm{F}_{\mathrm{ROH}_i}\mathrm{midLife}_i\beta_8 + \mathrm{F}_{\mathrm{ROH}_i}\mathrm{lateLife}_i\beta_9 + \alpha_j^{\text{capture year}} + \\ \alpha_k^{\text{birth year}} + \alpha_l^{\mathrm{id}} + u_l^{\mathrm{ped}})\end{aligned} \quad (1)$$

535

536 $$\alpha_j^{\text{capture year}} \sim N(0, \sigma^2_{\text{year}}), \qquad for\ j = 1, \dots, 40$$
537 $$\alpha_k^{\text{birth year}} \sim N(0, \sigma^2_{\text{birth year}}), \qquad for\ k = 1, \dots, 40$$
538 $$\alpha_l^{\mathrm{id}} \sim N(0, \sigma^2_{\mathrm{id}}), \qquad for\ l = 1, \dots, 5925$$
539 $$u_l^{\mathrm{ped}} \sim N(0, A\sigma_A^2) \qquad for\ l = 1, \dots, 5925$$
540

541 Here, $\Pr(\mathrm{surv}_i = 1)$ is the probability of survival for observation *i*, which depends on the intercept β_0,
542 a series of fixed effects β_1 to β_9, the random effects α which are assumed to be normally distributed
543 with mean 0 and variance σ^2 and the breeding values u_l^{ped} which have a dependency structure
544 corresponding to the pedigree, with a mean of 0 and a variance of $A\sigma_A^2$, where A is the relationship
545 matrix and σ_A^2 is the additive genetic variance. We used a pedigree-derived additive relatedness
546 matrix for computational efficiency as has previously been described for INLA models [80]. Variance
547 component estimates for additive genetic effects have also previously been shown to be very similar
548 when using a pedigree and a SNP derived relatedness matrix in Soay sheep [69]. Also, additive genetic
549 variance in Soay sheep fitness components is very low (Supplementary Table 4 and [71,81]). As fixed
550 effects, we fitted the individual inbreeding coefficient F_{ROH} (continuous), the lifestage of the
551 individual (categorical predictor with four levels: lamb (age=0, reference level), early life (age=1,2),

552 mid-life (age=3,4), late life (age=5+), sex (0 = female, 1 = male), and a variable indicating whether
553 an individual was born as a twin (0 = singleton, 1 = twin). We also fitted an interaction term of F_{ROH}
554 with lifestage to estimate how inbreeding depression changes across the lifetime. As lifestage was
555 fitted as a factor, the model estimates three main effects for the differences in the log-odds of survival
556 between lambs (reference category) and early life, mid-life and late life respectively. Similarly, the
557 interaction term between F_{ROH} and lifestage estimates the difference in the effect of F_{ROH} on survival
558 (i.e. inbreeding depression) between lambs and individuals in early life, mid-life and late life,
559 respectively. As random intercepts we fitted the birth year of an individual, the observation year to
560 account for survival variation among years and the sheep identity to account for repeated measures.
561 For all random effects, which are estimated in terms of precision rather than variance in INLA, we
562 used log-gamma priors with both shape and inverse-scale parameter values of 0.5. Supplementary
563 Table 4 gives an overview over the coding and standardization of the predictors.
564 Before modelling, we mean-centred [82,83] and transformed F_{ROH} from its original range 0-1 to 0-10,
565 which allowed us to directly interpret model estimates as resulting from a 10% increase in genome-
566 wide IBD rather than the difference between a completely outbred and a completely inbred
567 individual. Finally, we report model estimates as odds-ratios in the main paper, and on the link scale
568 (as log-odds ratios) in the Supplementary Material.
569
570 **Estimating lethal equivalents.**
571 The traditional way of comparing inbreeding depression among studies is to quantify the inbreeding
572 load in terms of lethal equivalents, i.e. a group of genes which would cause on average one death if
573 dispersed in different individuals and made homozygous [58]. However, differences in statistical
574 methodology and inbreeding measures make it difficult to compare the strength of inbreeding
575 depression in terms of lethal equivalents among studies [59]. Here, we used the approach suggested
576 by Nietlisbach et al. (2019) and refitted the survival animal model with a Poisson distribution and a
577 logarithmic link function. We were interested in lethal equivalents for the overall strength of
578 inbreeding depression rather than its lifetime dynamics, so we fitted a slightly simplified animal
579 model with F_{ROH}, age, age^2, twin and sex as fixed effects and birth year, capture year, individual id
580 and pedigree relatedness as random effects. The slope of F_{ROH} estimates the decrease in survival
581 due to a 10% increase in $F_{ROH,}$ so we calculated the number of diploid lethal equivalents 2B as –
582 $(\beta_{FROH})/0.10 * 2$ where β_{FROH} is the Poisson model slope for F_{ROH}.
583
584
585

586 **Mapping inbreeding depression.**

587 To map the effects of inbreeding depression in survival across the genome, we used a modification
588 of a genome-wide association study [47,48,84]. For each of the ~417K SNPs, we fitted a binomial mixed
589 model with logit link in lme4 [76] with the following model structure:

590

$$\Pr(\text{surv}_i = 1) = \text{logit}^{-1}(\beta_0 + \text{SNP}_{\text{ROH}_{\text{alleleA}_i}}\beta_1 + \text{SNP}_{\text{ROH}_{\text{alleleB}_i}}\beta_2 + \text{SNP}_{\text{ADD}_i}\beta_3 + \text{F}_{\text{ROH}_{\text{mod}_i}}\beta_4 + \text{age}_i\beta_5 + \text{age}_i^2\beta_6 + \text{sex}_i\beta_7 + \text{twin}_i\beta_8 + \beta_{9-16}\text{PC}_{1-7} + \alpha_j^{\text{capture year}} + \alpha_k^{\text{birth year}} + \alpha_l^{\text{id}}) \quad (2)$$

591

592 $\alpha_j^{\text{capture year}} \sim N(0, \sigma^2_{\text{capture year}}), \quad for\ j = 1, \dots, 40$

593 $\alpha_k^{\text{birth year}} \sim N(0, \sigma^2_{\text{birth year}}), \quad for\ k = 1, \dots, 40$

594 $\alpha_l^{\text{id}} \sim N(0, \sigma^2_{\text{id}}), \quad for\ l = 1, \dots, 5925$

595

596 Where the effects of interest are the two ROH status effects, $\text{SNP}_{\text{ROH}_{\text{alleleA}}}$ and $\text{SNP}_{\text{ROH}_{\text{alleleB}}}$. These are
597 binary predictors indicating whether allele A at a given SNP is in an ROH ($\text{SNP}_{\text{ROH}_{\text{alleleA}}}$ = 1) or not
598 ($\text{SNP}_{\text{ROH}_{\text{alleleA}}}$ = 0), and whether allele B at a given SNP is an ROH ($\text{SNP}_{\text{ROH}_{\text{alleleB}}}$ = 1) or not ($\text{SNP}_{\text{ROH}_{\text{alleleB}}}$
599 = 0). These predictors test whether an ROH has a negative effect on survival, and whether the
600 haplotypes containing allele A or the haplotypes containing allele B are associated with the negative
601 effect and therefore carry the putative recessive deleterious mutation. SNP_{ADD} is the additive effect
602 for the focal SNP (0,1,2 for homozygous, heterozygous, homozygous for the alternative allele,
603 respectively), and controls for the possibility that a potential negative effect of ROH status is simply
604 an additive effect. $\text{F}_{\text{ROH}_{\text{mod}}}$ is the mean inbreeding coefficient of the individual based on all
605 chromosomes except for the chromosome where the focal SNP is located. We fitted F_{ROH} as we were
606 interested in the effect of ROH status at a certain locus on top of the average individual inbreeding
607 coefficient. Sex and twin are again binary variables representing the sex of the individual and
608 whether it is a twin. Age and age^2 control for the linear and quadratic effects of age, and are fitted as
609 continuous covariates. Because it was computationally impractical to fit 417K binomial animal
610 models with our sample size and because the additive genetic variance in survival was very small
611 (posterior mean [95%CI] = 0.29 [0.22, 0.37], see Supplementary Table 4), we did not fit an additive
612 genetic effect. Instead, we used the top 7 principal components of the variance-standardized
613 additive relationship matrix ($\text{PC}_{1\text{-}7}$) as fixed effects [74]. Again, we added birth year, capture year and
614 individual id as random effects. For each fitted model, we extracted the estimated slope of the two
615 SNP_{ROH} predictors and their p-values, which were calculated based on a Wald-Z tests. To determine
616 a threshold for genome-wide significance we used the 'simpleM' procedure [85]. The method uses

617 composite linkage disequilibrium to create a SNP correlation matrix and calculates the effective
618 number of independent tests, which was much lower in than the number of SNPs (n_{eff} = 39184)
619 because LD stretches over large distances in Soay sheep (Supplementary Figure 9). We then
620 doubled this number to 78368, as we conducted two tests per model, and used this value for a
621 Bonferroni correction [86] of p-values, resulting in a genome-wide significance threshold of $p < 6.38 *$
622 10^{-7}.
623

624 **Data availability**

625 All data are available on Zenodo (http://doi.org/10.5281/zenodo.4609701) [87]. Source data are
626 provided with this paper.
627

628 **Code availability**

629 Code was written in R 3.6.1 [88] and relied heavily on tidyverse 1.3.0[89], data.table 1.14.0[90], and
630 ggplot2 3.3.3 [91]. The full analysis code is available on Zenodo
631 (http://doi.org/10.5281/zenodo.4587676)[92] and GitHub (https://github.com/mastoffel/sheep_ID).
632

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648
649

Author contributions

J.M.P. and M.A.S. designed the study. J.G.P. is the main Soay sheep project fieldworker and collected samples and life history data. J.M.P. has run the long-term Soay sheep study and organised the SNP genotyping. S.E.J. built the core genomic database, including genotyping, quality control and linkage mapping. M.A.S. conducted data analyses and drafted the manuscript. M.A.S., J.M.P. and S.E.J. jointly contributed to concepts, ideas and revisions of the manuscript.

Competing interests

The authors declare no competing interests.

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