Received: 5 December 2023 | Revised: 1 March 2024 | Accepted: 20 March 2024

DOI: 10.1111/mec.17335

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Genetic architecture of inbreeding depression may explain its persistence in a population of wild red deer

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Funding information
Royal Society, Grant/Award Number: UF150448; European Research Council; Natural Environment Research Council, Grant/Award Number: NE/S007407/1

Handling Editor: David Coltman

Abstract

Inbreeding depression is of major concern in declining populations, but relatively little is known about its genetic architecture in wild populations, such as the degree to which it is composed of large or small effect loci and their distribution throughout the genome. Here, we combine fitness and genomic data from a wild population of red deer to investigate the genomic distribution of inbreeding effects. Based on the runs of homozygosity (ROH)-based inbreeding coefficient, F_{ROH}, we use chromosome-specific inbreeding coefficients (F_{ROHChr}) to explore whether the effect of inbreeding varies between chromosomes. Under the assumption that within an individual the probability of being identical-by-descent is equal across all chromosomes, we used a multi-membership model to estimate the deviation of F_{ROHChr} from the average inbreeding effect. This novel approach ensures effect sizes are not overestimated whilst maximising the power of our available dataset of >3000 individuals genotyped on >35,000 autosomal SNPs. We find that most chromosomes confer a minor reduction in fitness-related traits, which when these effects are summed, results in the observed inbreeding depression in birth weight, survival and lifetime breeding success. However, no chromosomes had a significant detrimental effect compared to the overall effect of inbreeding, indicating no major effect loci. We conclude that in this population, inbreeding depression is likely the result of multiple mildly or moderately deleterious mutations spread across all chromosomes, which are difficult to detect with statistical confidence. Such mutations will be inefficiently purged, which may explain the persistence of inbreeding depression in this population.

KEYWORDS
inbreeding, inbreeding depression, quantitative genetics, runs of homozygosity

1 | INTRODUCTION

The reduced fitness of inbred individuals, known as inbreeding depression, is of particular concern for the management and conservation of declining species. Accumulation of inbreeding depression effects can lead to reduced population growth and, in the worst cases, extinction (Hedrick & Kalinowski, 2000; Keller & Waller, 2002). A number of wild populations across a range of taxa

have been reported to experience inbreeding depression in various fitness-related traits including fecundity, survival, body weight, germination success and susceptibility to disease. (e.g. Crnokrak & Roff, 1999; Hajduk et al., 2018; Keller & Waller, 2002; Liberg et al., 2005). Moreover, the overall cost of inbreeding depression is likely higher in wild free-living populations than for those in captivity (Ralls et al., 1988). Along with the increased risk of extinction, natural populations are more affected by environmental factors such as extreme weather, food scarcity, or disease, all of which will influence an individual's probability of survival (Crnokrak & Roff, 1999).

The two main hurdles to overcome in order to estimate inbreeding depression in natural populations are obtaining measures of fitness and obtaining a detailed pedigree from which to estimate meaningful individual inbreeding coefficients (Pemberton, 2008). Advances in genomic technologies, such as SNP arrays and whole-genome sequencing, have helped overcome the latter issue, instead enabling more precise marker-based estimations of inbreeding (Hedrick & Garcia-Dorado, 2016; Kardos et al., 2015). A handful of studies of non-model organisms living in the wild have since shown evidence for inbreeding depression using genomic data (e.g. Bérénos et al., 2016; Hoffman et al., 2014; Huisman et al., 2016; Kardos et al., 2023; Khan et al., 2021; Stoffel, Johnston, et al., 2021). Suitable data from wild populations can still be hard to obtain, particularly for large samples of individuals, but rising affordability of genomic data should increase the prevalence of these types of studies (Kardos et al., 2016). The unique value of genomic studies is the ability to uncover information on the genetic architecture of inbreeding depression, such as identifying loci with large effects and determining the genomic distribution of effects (Hedrick & Garcia-Dorado, 2016).

Genomic data may further uncover the extent to which recessive deleterious alleles or overdominant loci contribute to inbreeding depression in wild populations, which is still largely unknown (Kardos et al., 2016). Inbreeding increases homozygosity in the population, which, in both cases, leads to an increased frequency of the less fit genotype, either due to expressing the deleterious effect or due to not expressing the heterozygote phenotype respectively. Experimental evidence supports the existence of both these mechanisms but implies that overdominance is rare and of lesser importance to inbreeding depression than recessive deleterious mutations (Charlesworth & Willis, 2009; Falconer & Mackay, 1996). Slightly counterintuitively, severe inbreeding can reduce inbreeding depression by facilitating purifying selection, purging deleterious recessive mutations from the population. Theory shows that purging is faster and more effective for large-effect mutations as selection is stronger against these mutations, whereas the effects of inbreeding depression that are the result of multiple recessive small effect loci will be less easily purged (Charlesworth & Willis, 2009).

In our study population of red deer inhabiting the Isle of Rum, inbreeding depression has previously been demonstrated in several fitness-related traits (Coulson et al., 1998; Coulson et al., 1999; Slate et al., 2000; Walling et al., 2011), most recently by Huisman et al. (2016), who used individual inbreeding coefficients estimated from a SNP-derived genomic relatedness matrix. Huisman et al. (2016) showed inbreeding depression in birth weight, juvenile survival and lifetime breeding success (LBS) in both sexes. For an individual which is a product of a half-sibling mating (F≈0.125) LBS was decreased by 95% and 72% in males and females respectively (Huisman et al., 2016). Since this study, we have obtained precise genomic locations of SNPs through an assembly of the red deer (*Cervus elaphus*) genome (Pemberton et al., 2021), allowing for the identification of runs of homozygosity (ROH) (Hewett et al., 2023). ROH are a direct consequence of identity-by-descent in the genome and, depending on the density of genomic markers available, can allow for fine-scale measures of inbreeding and localisation of causal loci. For example, for a simple monogenic disease, a causal recessive mutation can be identified based on the presence or absence of disease in relation to segments of ROH (Kijas, 2013). More recently, ROHs have been used to identify genomic regions containing large effect loci in a range of complex traits (Hill et al., 2022; Pryce et al., 2014; Stoffel, Johnston, et al., 2021), highlighting the value of using ROHs in this type of analysis. Here, given the density of SNP genotypes available, our aim was to use ROH to identify whether specific chromosomes underpin inbreeding depression in this wild population of red deer. As a consequence of independent segregation and recombination, there is potential for different chromosomes to vary in their inbreeding effects, but at the same time, they are not entirely independent. Under this reasoning, we used a modified version of the ROH-based inbreeding coefficient (F_{ROH}) to quantify chromosome-specific inbreeding coefficients (F_{ROHChr}), and tested whether any chromosomes disproportionately contribute to inbreeding depression using multi-membership models.

2 | METHODS

2.1 | Genotyping and ROH calling

DNA was extracted from ear punches, post mortem tissues and cast antlers (see Huisman et al. [2016] for details) for 3198 individuals living within the red deer study population on the Isle of Rum between 1958 and 2022. Genotyping of DNA samples was performed on the Cervine Illumina 50 K BeadChip (Brauning et al., 2015). SNP genotypes were clustered and scored using Illumina GenomeStudio v2.0 and the following quality control was imposed: SNP minor allele frequency (MAF) >0.001, ID genotyping success >0.9, and SNP genotyping success >0.99; leading to 39,587 SNPs being retained at this stage. SNPs were mapped to the red deer (*C. elaphus*) genome assembly mCerEla1.1 (Pemberton et al., 2021).

We used the *--homozyg* function in *PLINK* v2.0 (Chang et al., 2015; Purcell et al., 2007) to search for ROH in the population. The following parameters were used to identify ROH using a 35 SNP sliding window: 40 SNPs as the minimum number of SNPs in a ROH, minimum length of a ROH 2500 kb, minimum density of 1 SNP per 70 kb, 4 missing SNPs allowed in a window, 0 heterozygote SNPs allowed, and a minor allele frequency threshold of 0.01. A total of 36,997 mapped SNPs passed automatic filter and quality controls

imposed by the *PLINK* parameters used and all individuals passed quality controls.

2.2 | Chromosome-specific inbreeding coefficient

Chromosome-specific inbreeding coefficients (F_{ROHchr}) for each individual were analogous to the genome-wide inbreeding coefficient, F_{ROH}, where F_{ROH} is the sum of Mb in a ROH divided by the total autosome size. For every individual, we summed the total Mb in a ROH on each autosome, then divided this by the corresponding chromosome length in Mb:

$$F_{ROHchr} = \frac{\text{Mb in a ROH}}{\text{Chromosome length (Mb)}} \quad (1)$$

Chromosome lengths were obtained from the red deer genome assembly, mCerEla1.1 (Pemberton et al., 2021).

2.3 | Study traits

2.3.1 | Estimated birth weight (kg)

We used neonatal capture weight in kg as a proxy for calf weight at birth. Calves are sampled and weighed soon after birth and their age in hours is also estimated based on field observations. In this model we fitted both the mother's ID and an inverse pedigree relatedness matrix (ARM) to account for known maternal effects and additive genetic variance (Gauzere et al., 2020 and Gauzere et al., 2022), see Table 1 for additional covariates. We fitted the ARM rather than a GRM to speed up modelling and in the knowledge that estimates of additive genetic variance were likely to be very similar (Bérénos et al., 2014).

2.3.2 | Juvenile survival (age 0–2 years)

Juvenile survival was considered as a binary response variable, that is, either 0 or 1. Any individual that died before the month of June 2 years after the year of birth was assigned a juvenile survival value of 0. Any individual that survived this time period was assigned a juvenile survival of 1. For most individuals, an accurate death date is known from regular censuses throughout the year and mortality searching in winter, allowing for easy calculation of juvenile survival. For individuals with no known death information, an estimated death year was assigned when the individual had not been sighted for 3 years. Individuals that died due to accidents, desertion following tagging or being shot after ranging outside the study area before 2 years of age were removed from the dataset. Individuals that were shot as adults when ranging outside the study area were recorded as having a juvenile survival of 1.

For this and all subsequent traits, no inverse relatedness matrix was fitted as previous studies show they have low heritability (Gauzere et al., 2020). Since there is inbreeding depression in birth weight, during modelling of juvenile survival we ran models with and without estimated birth weight (kg) fitted as a fixed effect to check the extent to which any inbreeding depression in juvenile survival was due to inbreeding depression in birth weight. This had no effect on the mean posterior estimates of F_{ROHchr} (though it did widen the confidence intervals, see Figure S3). The final model presented does

TABLE 1 Model details for the investigated traits.

Trait	Birth weight	Juvenile survival	Female LBS	Male LBS
Model family	Gaussian	Threshold	Hurdle Poisson	Zero inflated Poisson
Fixed effects	Sex; Estimated age in hours; Mother status at time of birth[a]; Mother age at time of birth; Mother age at time of birth^2	Sex; Mother status at time of birth[a]; Mother age at time of birth; Mother age at time of birth^2	None	None
Random effects	Mother ID; Birth year; Inverse pedigree relatedness matrix	Mother ID; Birth year	Mother ID; Birth year	Mother ID; Birth year
Iterations	100,000	300,000	650,000	1,000,000
Thinning interval	50	100	200	500
Burn-in	10,000	50,000	150,000	250,000
Sample size	2632	2737	692	641

Note: All models were run in MCMCglmm. For each trait column, rows show the model family type; fixed effects; variables treated as random effects; number of iterations the model was run for; thinning interval (i.e. the intervals at which the Markov chain is stored); the burn-in and the number of individuals in each input data frame.

[a]Levels: True yeld (did not give birth the previous year), Summer yeld (gave birth the previous year but the calf died over the summer), Winter yeld (gave birth the previous year but the calf died over the winter), Milk (gave birth the previous year and the calf survived the winter), Naïve (first-time breeder).

not include birth weight since it appears any inbreeding depression in birth weight is subsumed within inbreeding depression in juvenile survival.

2.3.3 | Female lifetime breeding success (LBS)

Females can conceive for the first time at age 2, producing their first calf at age 3 (i.e. succeeding in the juvenile survival period) and can continue reproducing into later life. Total female LBS was calculated for females that were born before 2006 that died of natural causes or were still alive in 2023 ($n = 10$), that is, near the end of their reproductive span. Female breeding success is known from close field observation and confirmed by pedigree reconstruction using the pedigree inference software SEQUOIA (Huisman, 2017). We used a hurdle Poisson process to model female LBS as if they reach reproductive age, most females will reproduce at least once during their lifetime. In this case, the 'hurdle' denotes survival to reproductive age and the truncated Poisson distribution denotes individuals with LBS > 0.

2.3.4 | Male lifetime breeding success (LBS)

Males can begin siring calves at ~5 years of age (i.e. 3 years after the juvenile survival period) but are often not competitive after 10 years of age. Total LBS was calculated for all males that rutted in the study area and died of natural causes. We omitted records for all individuals born from 2008 onwards to avoid biasing results towards males that die young. Males can continue rutting until ~15 years of age, hence, any male born after 2008 may not have reached their total LBS. Paternity was previously assigned through pedigree reconstruction using SNP genotypes and the pedigree inference software SEQUOIA (Huisman, 2017). We used a zero-inflated Poisson process to model male LBS in the knowledge that in the highly competitive mating system, a male can have zero LBS as a result of two causes: death before reproductive age (represented through the zero inflation) and failure to breed successfully (captured in the Poisson sampling distribution).

2.4 | Statistical analysis of chromosome-specific inbreeding effects

All statistical models were run in the MCMCglmm R package (Hadfield, 2010). Our aim was to determine whether inbreeding depression is evenly distributed across chromosomes. We focussed on four traits outlined above with evidence for inbreeding depression identified from previous studies. We used a multi-membership approach to explore whether the effect of inbreeding varies between chromosomes for each trait, under the assumption that within individuals the F_{ROHchr} values are expected to be related as inbreeding is genome-wide. In this approach, the effect of each F_{ROHchr} is estimated as a deviation from the average effect of inbreeding. For each individual, inbreeding coefficients were summed across all chromosomes, which when fitted as a fixed effect describes the average effect of inbreeding among chromosomes. During model refinement, we also fitted genome-wide inbreeding coefficients (F_{ROH}) or the sum of F_{ROHChr} divided by the number of chromosomes as alternative methods to incorporate the overall effect of inbreeding on traits. These models yielded the same effect estimates, therefore, for interpretation and plotting purposes, we proceeded with the use of the summed F_{ROHChr}. By treating all F_{ROHchr} as random effects and estimating their deviation we ensured we did not overestimate the effect of individual $F_{ROHchrs}$.

For each trait, the chromosome-specific inbreeding depression in the ith individual was modelled as:

$$y_i = b_0 + b_1 \sum_{c=1}^{33} F_{ci} + \sum_{c=1}^{33} u_c F_{ci} \quad (2)$$

where, b_0 is the intercept and F_{ci} is the inbreeding coefficient for an individual i on chromosome c. b_1 is the estimated average effect of inbreeding and u_c is the deviation from the average effect for chromosome c. Trace plots were inspected for validation of model convergence. Specific model parameters including additional fixed and random effects for each trait are shown in Table 1.

We also repeated this analysis using a finer-scale inbreeding coefficient, where F_{ROHChr} was replaced with the inbreeding coefficient of 5 Mb non-overlapping windows ($F_{ROH\_5Mb\_window}$). We only performed this fine-scale model on birth weight and juvenile survival as these have the largest sample sizes and had the most variability in chromosome-specific effect sizes. We omitted the pedigree relatedness matrix from the model of birth weight to minimise the computing power required. Both models were run for 1 million iterations with a 250,000 burn-in. Results are shown in Figure S4.

We used the 95% confidence intervals of the posterior estimates to assess the significance of each F_{ROHchr}. Chromosomes where confidence intervals did not overlap the average effect of inbreeding were deemed significantly different. To calculate absolute F_{ROHchr} effects for each chromosome for trait predictions, we combined the estimated deviation of F_{ROHchr} with the average effect of inbreeding. Trait predictions where inbreeding occurs to the same degree on all chromosomes assume that F_{ROHchr} is the same on all chromosomes, effects are summed to get the overall effect of inbreeding on each trait. Trait predictions where chromosomes are treated as independent assume that the inbreeding coefficient is present only on the focal chromosome and all other chromosomes are outbred (i.e. $F_{ROHchr} = 0$).

2.5 | Variance explained by F_{ROHChr}

We calculated the partial R^2 (Nakagawa & Schielzeth, 2013; Stoffel, Nakagawa, et al., 2021), or variance explained, by each F_{ROHChr} using:

$$\frac{\beta_1^2 Var_1 + \beta_c^2 Var_c + 2\beta_1\beta_c cov(1,c)}{Var_{Total}} \quad (3)$$

where, β_1^2 is the estimated slope of the average effect of inbreeding squared and Var_1 is the variance in average inbreeding predictor. β_c^2 is the squared estimated deviation of the c^{th} chromosome estimated from the model and Var_c is the variance in the c^{th} F_{ROHChr} predictor, $cov(1, c)$ is then the covariance between average inbreeding and F_{ROHChr}. Finally, the total trait variance (Var_{Total}) is the sum of the variance explained by all fixed and random effects fitted in the model. The variance explained by F_{ROHChr} was then correlated with chromosome length in Mb using a simple linear model (y ~ x). Results are shown in Figure S2.

3 | RESULTS

The mean chromosome-specific inbreeding coefficient (F_{ROHchr}) was 0.064 (equal to the population mean genome-wide inbreeding coefficient, F_{ROH}) and ranged between 0 and 0.94, with the highest F_{ROHchr} found in two individuals on chromosome 3 (see outliers in Figure S1). F_{ROHchr} within individuals were generally weakly positively correlated, except for chromosomes 2 and 21, 7 and 13, 12 and 18, and 31 and 14 which were slightly negatively correlated, see Figure 1.

3.1 | Evidence for inbreeding depression

Overall, higher inbreeding coefficients were associated with significantly lower birth weights and probability of survival to age 2 (p-values <.001). A sex-averaged calf with a F_{ROHchr} of 0.25 on all chromosomes was predicted to weigh 6.14 kg (CIs: 5.83–6.43 kg) at birth, whereas, a completely outbred individual (F = 0) was predicted to weigh 7.08 kg (CIs: 6.91–7.25 kg), see Figure 2a. For the probability of survival, the equivalent predictions were 36% (CIs: 21%–54%) and 74% (CIs: 69%–80%), Figure 2b. We found a significant effect of inbreeding coefficient on whether a female 'crossed the hurdle' (i.e. had at least 1 offspring, p-value: 0.017), but no effect on the truncated Poisson process (number of offspring given the female had at least one offspring, p-value: .983). The combination of these processes predicts that a female with an inbreeding coefficient of 0.25 on all chromosomes has a LBS of 1.8 (CIs: 0.6–3.9) compared to a completely outbred female which has a predicted LBS of 4.6 (CIs: 3.6–5.6) (Figure 2c). In males, we found a marginally significant association of the overall inbreeding coefficient with the zero inflation element (p-value: .048) and a stronger association with the Poisson process (p-value <.001). The combined effect of both processes shows a male with an inbreeding coefficient of just 0.1 is barely predicted to have one offspring, (LBS prediction:0.8, CIs: 0.3-1.8), whereas a completely outbred male is predicted to have a total of 11.4 (CIs: 6.2-19.4) offspring (Figure 2d).

3.2 | Chromosome-specific effects of inbreeding

Across all four tested traits, the majority of chromosomes showed a marginal decrease in fitness with increasing F_{ROHchr} (Figures 3 and 4), but no chromosome had a significantly detrimental effect compared to the average effect of inbreeding (Figure 3). When individual chromosome effects are summed, they produce the overall inbreeding depression shown in Figure 2. One exception is shown for inbreeding on chromosome 12 (highlighted red in Figure 3b) which was significantly associated with an increase in survival (although the lower

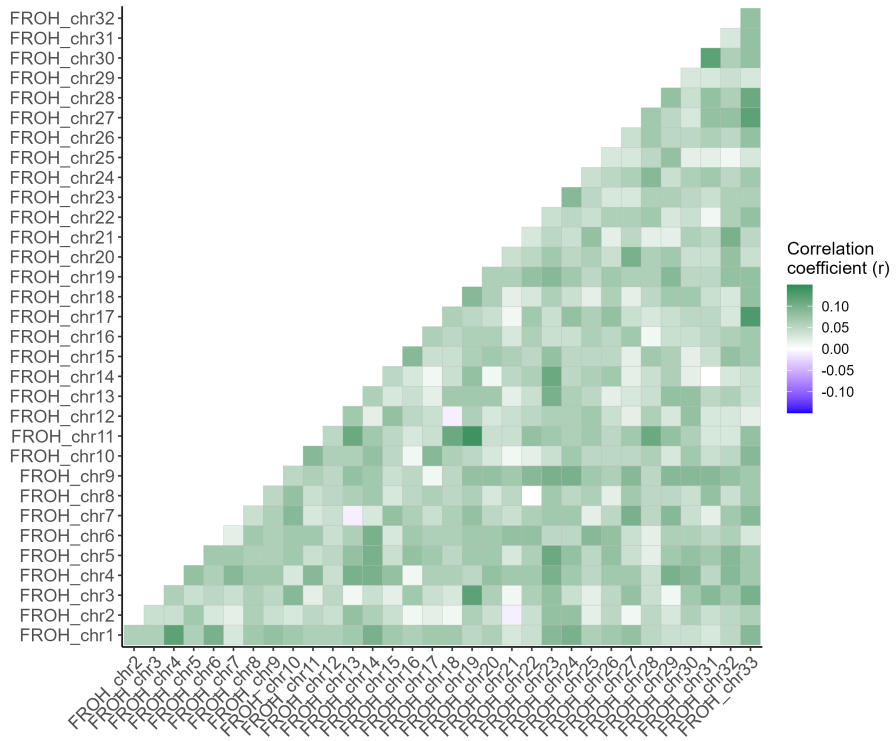

FIGURE 1 Correlation matrix of all chromosome-specific inbreeding coefficients (F_{ROHchr}) within individuals. Green shows a positive correlation between F_{ROHchr}, with stronger correlations shown in darker green, up to a maximum Pearson's correlation coefficient of 0.15. Negative correlations are shown in purple, with darker purple showing stronger negative correlations up to a minimum of Pearson's correlation coefficient of −0.15.

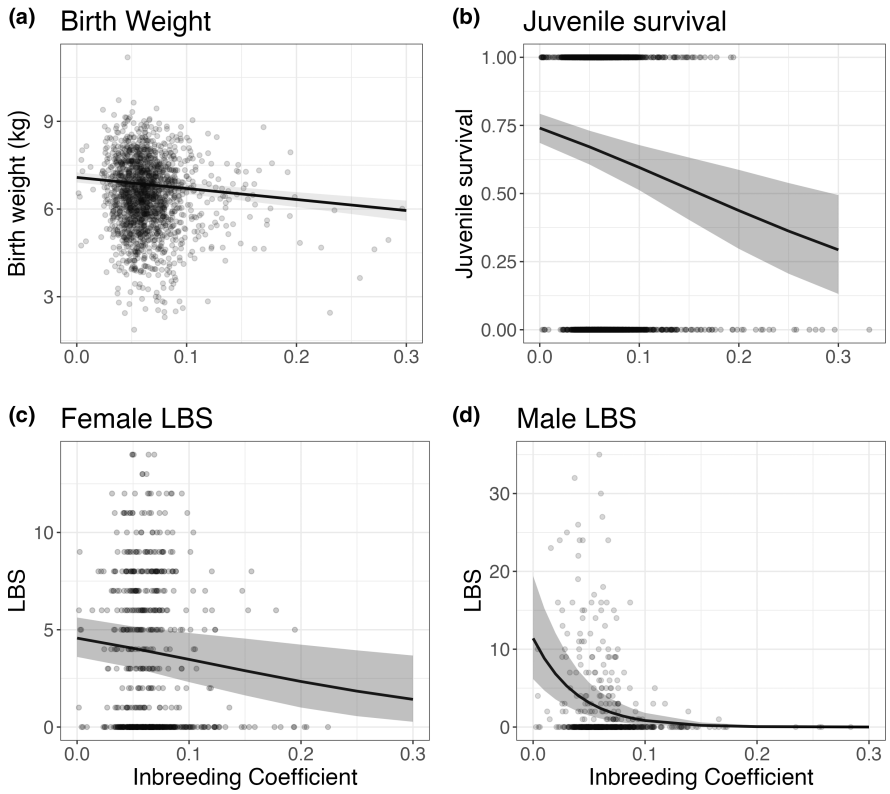

FIGURE 2 Predicted birth weight (a), juvenile survival probability (b) and lifetime breeding success in females (c) and males (d) using model estimates for increased inbreeding coefficient, where F_{ROHChr} is the same value on all chromosomes. Solid lines show the mean posterior estimate for an average individual and the grey area shows the 95% confidence intervals for the posterior estimate based on stored iterations. For figures c and d, predictions use the combined processes of hurdle/zero-inflation and truncated Poisson/Poisson. Points show the raw data. For figure a, capture weights are only shown for individuals <48 h old at capture for plotting purposes.

95% confidence interval nearly touches the average). Increased F_{ROHchr} on chromosome 12 predicts a modest increase in survival rate, whereas, for all other chromosomes, it predicts a lower rate of survival (Figure 4).

3.3 | Variance explained and chromosome length

For birth weight, the variance explained by inbreeding was positively correlated with chromosome length in a simple linear correlation (p-value = .044, R = 0.35, Figure S2). None of the other traits show this pattern, which may indicate that some chromosomes have a greater (or smaller) effect than expected given their size. For example, F_{ROHChr} for chromosome 13 explained the second greatest proportion of variance in juvenile survival despite being of intermediate length (Figure S2b).

F_{ROHChr} for chromosome 12, which was associated with an increase in juvenile survival (Figures 3 and 4), explains the lowest proportion of variance (Figure S2b). This can be attributed to the inclusion of the covariance between F_{ROHChr} and average inbreeding (Equation 3), which will account for the fact that being more inbred on chromosome 12 means having higher inbreeding overall.

3.4 | Fine-scale measurements of inbreeding depression

Implementing the multi-membership approach on 5 Mb windows yielded similar conclusions as above. Windows within chromosomes varied in effect size, although credible intervals were greatly increased compared to chromosome-specific estimates and all overlapped the average inbreeding effect, see Figure S4.

4 | DISCUSSION

The distribution and effect size of loci underpinning inbreeding depression in wild populations using fitness data has seldom been investigated. Our main aim was to gain a better understanding of the genetic architecture of inbreeding depression in the population of red deer inhabiting the Isle of Rum. In order to do so, we estimated the inbreeding effect size on individual chromosomes for traits previously identified as experiencing inbreeding depression. Using a larger sample size, we confirmed previous estimations of inbreeding depression for birth weight, juvenile survival and lifetime breeding success (Huisman et al., 2016). On average, calves produced from a full-sibling or parent-offspring pairing ($F_{ROH} \approx 0.25$) were ~12% lighter at birth and had a ~38% lower probability of survival than a completely outbred calf. During the refinement of the model of juvenile survival, we also fitted birth weight as an explanatory variable but found no difference in the mean effect estimates. As a result, we chose to omit it from the final model as inbreeding depression in birth weight appears to be a component of inbreeding depression in juvenile survival.

We also replicate findings of inbreeding depression in lifetime breeding success (LBS) found in Huisman et al. (2016), where inbred individuals of both sexes were less likely to have at least one offspring. Given the knowledge of inbreeding depression on

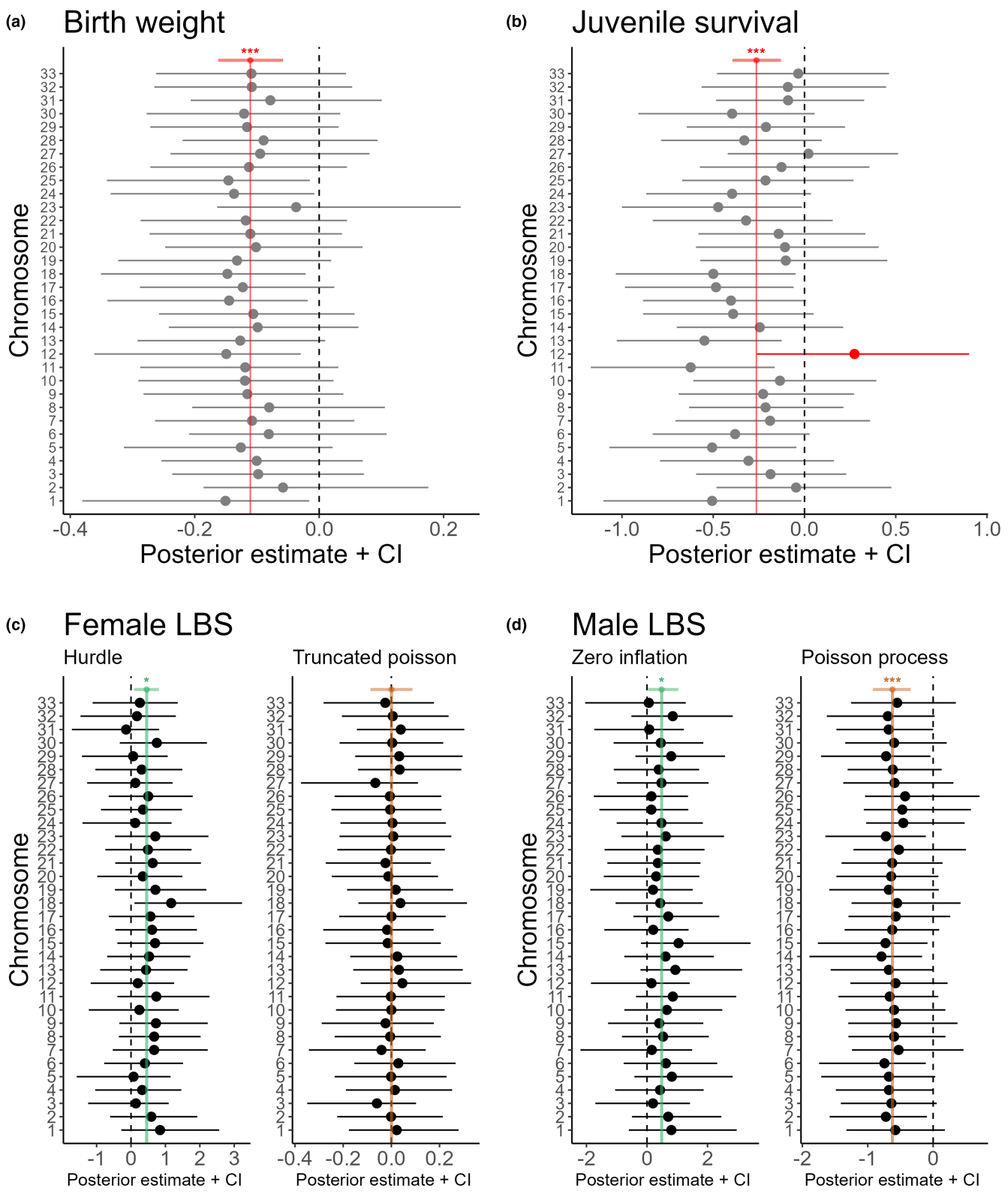

FIGURE 3 Deviation of chromosome-specific inbreeding coefficients from the summed inbreeding effect of all chromosomes for birth weight (a), juvenile survival (b) and lifetime breeding success for females (c) and males (d). Vertical dashed line shows H_0 (no effect of inbreeding). Figures c and d show the hurdle/zero-inflation on the left and the truncated Poisson/Poisson process on the right. Vertical solid red line in a and b shows the estimated effect of summed chromosomal inbreeding coefficients on trait. Vertical solid green lines show the mean estimated effect of summed chromosomal inbreeding coefficients on the hurdle (c) and zero inflation (d) expressed on the logit scale as the probability that LBS = 0, where a positive estimate indicates an increase in this probability. Vertical orange lines show the average inbreeding effect on the truncated Poisson (c)/Poisson (d). Horizontal coloured line shows the 95% credible intervals for the summed estimate and associated significance value (*: *p*-value <.05, ***: *p*-value <.001). Grey/black dots show the chromosome-specific point estimates and horizontal lines show the 95% confidence interval. Figure b chromosome 12 is highlighted in red as confidence intervals do not overlap the summed effect.

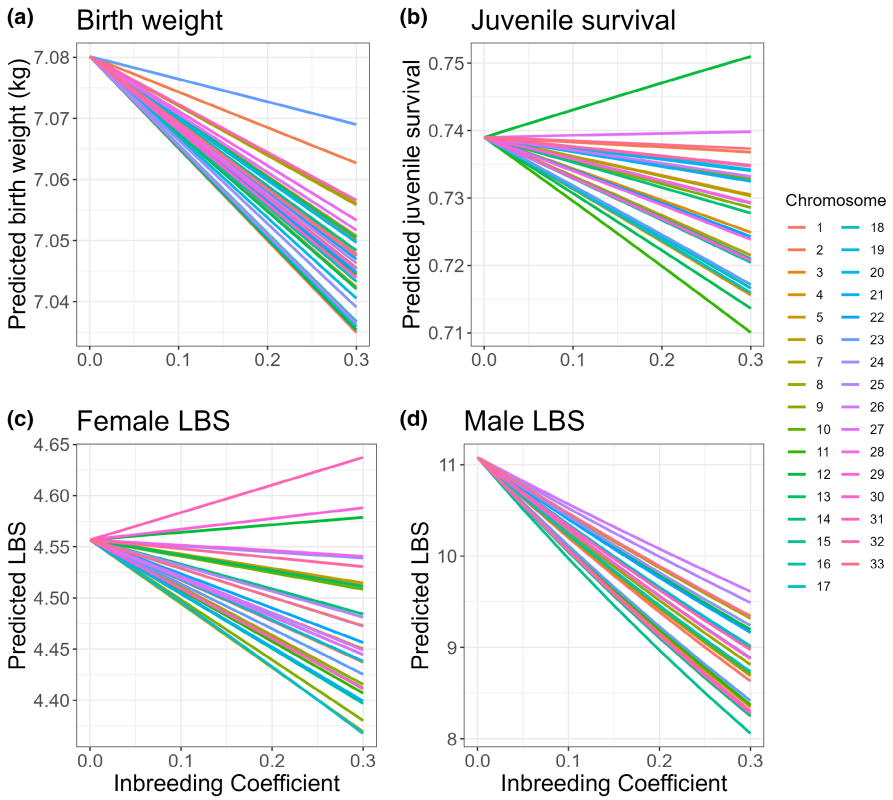

FIGURE 4 Mean predicted birth weight (a), juvenile survival (b) and lifetime breeding success for females (c) and males (d) for increasing F_{ROHChr} when chromosomes are treated independently in a hypothetical scenario, that is, F_{ROHchr} only increases on the focal chromosome and all other $F_{ROHchr}=0$. Most chromosomes show a minor reduction in fitness on the y axis.

juvenile survival, this result is perhaps expected. Nearly all females that survive to age 3 years reproduce (Pemberton et al., 2022). Therefore, most females that have no offspring are those that did not survive to reproductive age. For males, a large number of individuals do not sire any offspring either because they die before reproductive age or because they are unsuccessful in their reproductive efforts (Pemberton et al., 2022). A high proportion of males modelled in the zero-inflation process will then be individuals that do not survive to adulthood. Therefore, the inbreeding depression in LBS is partly influenced by the effects of inbreeding on juvenile survival. We further show that inbred males also had a lower number of total offspring compared to outbred males, analogous to Huisman et al. (2016). This suggests that inbred male are less competitive. Competition for matings with females is intense in red deer and the fighting success of a male is dependent on his size, weight and condition, which in turn plays a major role in whether he secures a harem and mates successfully (Clutton-Brock et al., 1982). Inbred males may be in worse condition, less likely to win fights and therefore unlikely to hold large harems and sire multiple offspring. Alternatively, the lower number of offspring may be caused by the residual effects of juvenile survival as individuals with an LBS of zero captured in the Poisson process may be a mix of those that do not survive to adulthood and those that achieve no successful matings. Therefore, the effect on inbreeding depression in juvenile survival may be represented in both the zero inflation and the Poisson process.

Although the pedigree-based expectation of identity-by-descent is the same for all chromosomes within an individual, the correlations between chromosome-specific inbreeding coefficients within individuals were relatively low here, due to recombination and independent segregation (Figure 1). This is consistent with the overall low level of inbreeding in the population and the relatively low estimated identity disequilibrium (Huisman et al., 2016). Across all investigated traits, we found no chromosomes that have a significantly detrimental effect in comparison to the overall effect of inbreeding, similarly, when the same approach is taken with 5 Mb windows, no windows pass the significance threshold. Most chromosomes do show a marginal decrease in fitness with increased chromosomal inbreeding, which, when summed, results in the observed overall inbreeding depression. This indicates that in this population there are no chromosomes or regions that contribute disproportionately to the observed inbreeding depression, instead inbreeding depression is the cumulative result of many small effect loci spread across all chromosomes.

It is important to note that increased statistical power (through increasing the number of individuals and/or using denser genomic markers) may uncover loci with contributions to inbreeding depression. As is the case with complex traits, increasing the number of loci and individuals usually increases the number of loci associated with the trait, each explaining a small amount of variance (Visscher et al., 2010; Yengo et al., 2022). Indeed, recent studies of wild populations with denser SNPs, higher individual inbreeding coefficients and/or more individuals, detected large effect loci contributing to inbreeding depression (Duntsch et al., 2023; Stoffel, Nakagawa, et al., 2021). However, increasing power in this way is obviously difficult in studies of wild populations and still does not guarantee the statistical power to detect loci with major deleterious effects. Loci with a lethal effect would be expected

to occur at low frequencies, hence, the power to detect them will be lower as there are few individuals carrying the causal allele (Kardos et al., 2016). Moreover, statistical power also depends on the inbreeding level in the population. In this population, extreme inbreeding is rare and the incidence of ROH is relatively low (Hewett et al., 2023). If only a small number of individuals have a ROH at a locus, then the power to detect the effect is reduced. This is further supported by our fine-scale analysis (Figure S4), which showed larger credible intervals than chromosome-specific estimates due to the low number of individuals with ROHs in a 5 Mb window, reducing the power of estimates. Hence, even with an increase in power, our conclusions of inbreeding depression being due to many loci with modest/weak effects spread genome-wide would likely remain. Moreover, in birth weight—a normally distributed trait known to be highly polygenic (Gauzere et al., 2023)—we further show the variance explained by F_{ROHChr} was correlated with the length of the chromosome (Figure S2). Analogous to genomic partitioning of additive genetic effects in complex traits, this suggests that longer chromosomes contribute more to inbreeding depression because they contain more mutations. (Robinson et al., 2013; Visscher et al., 2007; Yang et al., 2011). However, we did not find this pattern in any other traits (Figure S2). While this could indicate scattered large effect loci causing inbreeding depression, an alternative interpretation is that our data is underpowered for these traits, which have non-normal distributions, and in the case of LBS, relatively low sample sizes.

If inbreeding depression in the red deer on Rum is caused by many loci with small effects, this could partly explain its persistence. The efficiency of purging deleterious loci depends on a number of factors including the selection coefficient and the rate of inbreeding. Mildly deleterious recessive mutations are not exposed to such extreme purifying selection and therefore are not easily purged (Charlesworth & Willis, 2009; Wang et al., 1999). In contrast, mutations with high selection coefficients (lethal or extremely detrimental fitness effects) will be quickly purged from populations. Therefore, if inbreeding depression is caused by loci with modest or weak effects in this population, selection may not be effective in removing the contributing alleles, although this does not mean that mutations with major deleterious effects have not existed in the past. Rather, the lack of evidence for such mutations implies they may have already been purged from the population.

One major exception to the general pattern was found on chromosome 12. Increased inbreeding on this chromosome was associated with significantly increased juvenile survival. For example, if an individual had a chromosomal inbreeding coefficient of 0.3 on chromosome 12 and was completely outbred on all other chromosomes, their survival probability would increase by 1.2% compared to if the individual was outbred on all chromosomes (Figure 4b). This may represent an example of a beneficial recessive allele which when homozygous confers a survival advantage. In livestock species, such regions are often associated with economically important genes where being homozygous can result in an increased yield and are therefore usually under intense artificial selection (He et al., 2020). However, there are 909 protein-coding genes annotated on chromosome 12 in *C. elaphus* (mCerEla1.1), hence, there seems little benefit in hypothesising the mechanisms of selection acting here.

5 | CONCLUSIONS

In summary, we show evidence for ongoing inbreeding depression in birth weight, juvenile survival and lifetime breeding success in a wild population of red deer. Most chromosomes show a minor decrease in fitness with increased chromosome-specific inbreeding coefficients but, except for one instance discussed above, none are significantly different from the average effect of inbreeding over the whole genome. Using a finer-scale measure of inbreeding, we also show that while effect sizes vary within chromosomes the statistical confidence in the effect is reduced when using a finer scale, and consequently, as above, no regions pass the significance threshold. It is clear there is a trade-off between power, resolution and confidence in the effect size when identifying loci that explain inbreeding depression. In this population, inbreeding depression is probably the result of many loci each with a modest or weak effect, which are difficult to detect with statistical confidence and, as theory predicts, will be inefficiently purged leading to the persistence of inbreeding depression.

AUTHOR CONTRIBUTIONS

J.M.P. coordinated sampling and genotyping and supervised the fieldwork. A.M.H. conducted analyses and drafted the manuscript. A.M. and S.M. collected the data. J.M.P., A.M.H. and S.E.J. contributed to revisions.

ACKNOWLEDGEMENTS

We thank NatureScot for permission to work on the Isle of Rum. We thank the many field workers that have helped to collect samples and life histories on Rum. We are also grateful to Jarrod Hadfield, Joel Pick, Kirsty Macphie and Albert Phillimore for statistical advice and discussions. We also thank Martin Stoffel for discussions as well as Ino Curick and Simon Martin for discussions and comments on an earlier version of the manuscript. The long-term project and this research were funded by the UK Natural Environment Research Council, and most of the SNP genotyping was supported by a European Research Council Advanced Grant to J. M. Pemberton. A. Hewett was supported by an E4 NERC DTP studentship (NE/S007407/1). S.E. Johnston is supported by a Royal Society University Research Fellowship (UF150448). Genotyping was conducted at the Wellcome Trust Clinical Research Facility Genetics Core. We are grateful to the Darwin Tree of Life project for the physical map positions used.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests in relation to the work described.

DATA AVAILABILITY STATEMENT

All R scripts are available at: https://github.com/annamayh/GeneticArch_ID_Deer. Data used are available as a FigShare project: Hewett, Anna (2024). survival_df_anon.txt. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25053233.v1. Hewett, Anna (2024). ped_anon.txt. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25053941.v1. Hewett, Anna (2024). Female_LBS_df_anon.txt. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25053236.v1. Hewett, Anna (2024). male_LBS_df_anon.txt. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25053239.v1. Hewett, Anna (2024). birth_weight_df_anon.txt. figshare. Dataset. https://doi.org/10.6084/m9.figshare.25053230.v1.

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How to cite this article: Hewett, A. M., Johnston, S. E., Morris, A., Morris, S., & Pemberton, J. M. (2024). Genetic architecture of inbreeding depression may explain its persistence in a population of wild red deer. *Molecular Ecology*, *33*, e17335. https://doi.org/10.1111/mec.17335