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Inbreeding depression across the lifespan in a wild mammal population

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Inbreeding depression is of major concern for the conservation of threatened species, and inbreeding avoidance is thought to be a key driver in the evolution of mating systems. However, the estimation of individual inbreeding coefficients in natural populations has been challenging, and, consequently, the full effect of inbreeding on fitness remains unclear. Genomic inbreeding coefficients may resolve the long-standing paucity of data on inbreeding depression in adult traits and total fitness. Here we investigate inbreeding depression in a range of life history traits and fitness in a wild population of red deer (*Cervus elaphus*) in Scotland using individual inbreeding coefficients derived from dense Single-Nucleotide Polymorphism (SNP) data (F_{grm}). We find associations between F_{grm} and annual breeding success in both sexes, and between maternal inbreeding coefficient and offspring survival. We also confirm previous findings of inbreeding depression in birth weight and juvenile survival. In contrast, inbreeding coefficients calculated from a deep and comparatively complete pedigree detected inbreeding depression in juvenile survival, but not in any adult fitness component. The total effect of inbreeding on lifetime breeding success (LBS) was substantial in both sexes: for $F_{grm} = 0.125$, a value resulting from a half-sib mating, LBS declined by 72% for females and 95% for males. Our results demonstrate that SNP-based estimates of inbreeding provide a powerful tool for evaluating inbreeding depression in natural populations, and suggest that, to date, the prevalence of inbreeding depression in adult traits may have been underestimated.

fitness | adult traits | parental inbreeding | red deer | Single-Nucleotide Polymorphism

Decreasing fitness with increasing inbreeding is a widespread phenomenon (1), which occurs because mating between relatives increases homozygosity at loci carrying rare recessive deleterious alleles or exhibiting overdominance (2). The magnitude of inbreeding depression is relevant to many disciplines within biology, including the conservation of small, isolated populations (3), animal and plant breeding (4), trait variation in humans (5), and the evolution of mating systems (2, 6). In wild animal populations, evidence for inbreeding depression typically comes in the form of decreased juvenile survival (1, 6). The detrimental effects of inbreeding on adult traits such as fecundity, longevity, offspring birth weight, and milk production are well known in agricultural and zoo populations (e.g., refs. 4 and 7), but their prevalence and magnitude in wild populations remain hitherto unclear.

There is no a priori genetic or ecological reason to expect inbreeding depression to be reduced late in the life cycle, other than the higher opportunity for selection in juveniles (8). The lack of widespread evidence for adult inbreeding depression could partly be explained by the general acceptance that inbred individuals may be rare, due to inbreeding avoidance (6), and inbred adults even rarer when there is selection against inbred juveniles (1). More importantly, estimating inbreeding in natural populations is not trivial.

Pedigree-based inbreeding coefficients (F_{ped}), the traditional estimate of genome-wide homozygosity through identity by descent (IBD), are not available for most natural populations, as accurate and sufficiently deep pedigrees are difficult to construct. Short or incomplete pedigrees generate downward biased estimates of inbreeding coefficients, which decreases the power to detect inbreeding depression (9, 10). Bias may be reduced through exclusion of individuals with little or no ancestry information, such as founders, immigrants, and their offspring, but this can drastically reduce sample size (9). Consequently, relatively few studies of inbreeding depression in wild populations are based on pedigrees, although there are some notable exceptions (see ref. 1).

As an alternative to pedigrees, average homozygosity at genetic markers has been widely used to estimate an individual's inbreeding status. However, for many natural populations, genetic resources have hitherto been limited to small panels of markers, typically microsatellites, yielding estimates of marker homozygosity that are often poorly correlated with genome-wide homozygosity (9, 11). Consequently, correlations between microsatellite homozygosity and phenotypic trait values are typically small, although a meta-analysis showed an overall weak effect of decreasing fitness with increasing microsatellite homozygosity (12).

In recent years, genome-wide high-density marker data, such as that provided by panels of Single-Nucleotide Polymorphisms (SNPs), are becoming available for an increasing number of species. Theory and simulations predict that average homozygosity at a large number of SNPs provides a more precise estimate of genome-wide homozygosity than inbreeding coefficients from even a perfect pedigree, because it can capture the variation in IBD around the pedigree expectation, brought about by Mendelian segregation and recombination (13, 14). By chance, and because of physical linkage, some individuals with the same pedigree inbreeding coefficient (e.g., $F_{ped} = 0.25$ after a mating between full siblings) inherit a

Significance

Inbreeding depression is the decrease in fitness with increased genome-wide homozygosity that occurs in the offspring of related parents. Estimation of its effect in wild populations has been challenging, and while evidence of inbreeding depression in juvenile traits is widespread, examples during later life stages remain rare. Here, in a species with extended maternal care, genomic inbreeding coefficients, but not pedigree-based ones, revealed inbreeding depression in annual breeding success in both sexes, and in offspring rearing success in females. This contributed to inbreeding depression in estimates of lifetime fitness in both sexes. Our work illustrates that inbreeding depression in adult traits can be as large as in juvenile traits but requires more powerful methods to be detected.

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larger proportion of their alleles from the same ancestral copy than expected, including at causal loci. The performance of genomic relative to pedigree-based estimators of inbreeding and pairwise relatedness has been shown in simulations (14) and, among others, humans (5, 15) and cattle (16). The potential of large SNP panels in the wild was recently demonstrated in a small nonpedigreed sample of harbor seals (17), in which SNP heterozygosity showed a strong and highly significant association with parasite burden, whereas microsatellite heterozygosity did not.

However, in general it is not fully clear how novel SNP-based metrics of inbreeding will compare with the traditional pedigree-based measures in studies of wild populations. Their performance may not be as immaculate in real datasets as in simulations, as genomic estimators, like microsatellite markers, rely on a correlation in homozygosity between loci within individuals (9, 11). This so-called identity disequilibrium comes about through a fraction of systematic consanguineous matings, genetic drift, or admixture (9), but its magnitude can be difficult to predict.

Here, we combine dense SNP data with detailed life history data for a large number of individuals, to examine whether genomic estimates reveal inbreeding depression in fitness and various fitness components, in particular during adult life stages. We use a population of red deer (*Cervus elaphus*) in the North Block of the Isle of Rum, Scotland, which have been individually studied for over four decades. Our data are relatively rare in that they yield estimates of individual fitness (lifetime production of offspring), as well as various fitness components, and factors affecting each are well known (18, 19). Previous studies found inbreeding depression in birth weight and juvenile survival (20–22), as well as an association between microsatellite heterozygosity and lifetime breeding success (LBS) (23). In this species with extensive maternal care (18), an effect of the maternal inbreeding coefficient on offspring fitness has been hypothesized but not confirmed (22).

First, we compared genomic inbreeding coefficients with traditional pedigree-based inbreeding coefficients. Next, we tested for inbreeding depression in several fitness components and correlated traits, including juvenile survival, and annual survival and annual breeding success (ABS) in adults of both sexes, using both inbreeding measures. For traits expressed in juveniles, we considered the effects of both offspring and maternal inbreeding coefficients. Lastly, we investigated the cumulative effect of inbreeding on all traits, by considering the association between inbreeding coefficient and LBS in each sex, as well as with female lifetime reproductive success (LRS), the number of offspring which survived to independence.

Results

Level of Inbreeding. Based on the pedigree (details of which are given in *SI Materials and Methods* and Table S1), close inbreeding resulting in $F_{\mathrm{ped}} = 0.25$ is rare ($n = 10$ instances), and occurred only via father−daughter matings (in line with refs. 22 and 24). To minimize bias, F_{ped}-based analysis was restricted to individuals for whom it was, at minimum, clear whether or not they were the product of close inbreeding, i.e., for which at least both parents and the maternal grandfather were known. In this restricted dataset, 45% of individuals had an F_{ped} greater than zero (899/2,012), of which 125 individuals (6%) had $F_{\mathrm{ped}} \geq 0.05$.

Our genomic inbreeding estimator (F_{grm}, for Genomic Relatedness Matrix; details in *SI Materials and Methods* and Fig. S1) was strongly correlated with F_{ped} ($r = 0.74$, Fig. 1) and with average homozygosity ($r = 0.94$, Fig. S2). This estimator by Yang et al. (25) estimates how similar the gametes were that made up an individual's genome, relative to a random draw from a reference population (here: a random-mating population with the same allele frequencies as among our sampled individuals), and can take negative values (see Fig. 1). The distribution of F_{grm} is more convenient statistically than F_{ped}, as the high, narrow peak at $F_{\mathrm{ped}} = 0$ becomes an approximately normal distribution

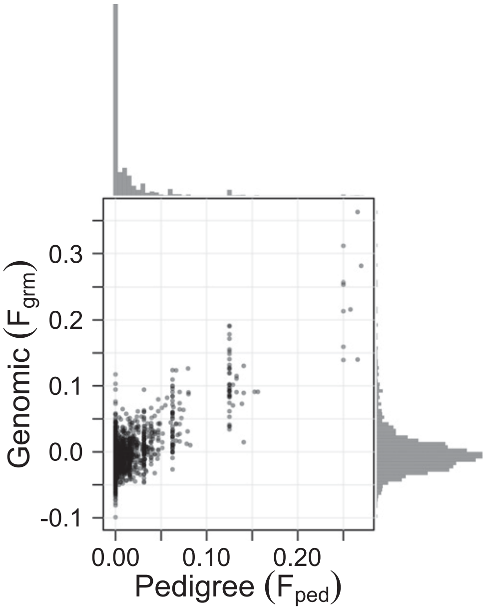

Fig. 1. Pedigree and genomic inbreeding coefficients for $n = 1{,}968$ individuals for whom both measures were available, including individuals with at least both parents and the maternal grandfather known for F_{ped} (see *Results, Level of Inbreeding*); $r = 0.74$, $\beta \pm \mathrm{SE} = 0.886 \pm 0.018$. Histograms show the distributions of F_{ped} (top) and F_{grm} (right). At $F_{\mathrm{ped}} = 0.125$, the scatter of F_{grm} is in line with the theoretical distribution of realized inbreeding coefficients for a mating between half-siblings [2.5–97.5 percentile: 0.05–0.20 (13); based on a human genome, which is of similar total length as the red deer genome].

centered at $F_{\mathrm{grm}} = 0$ (histograms in Fig. 1). The variation in F_{grm} within F_{ped} classes was expected (see the Introduction), and is due to both pedigree incompleteness and variation in realized genome-wide IBD around the pedigree expectation. Identity disequilibrium estimated from marker loci differed significantly from zero in the complete dataset [$g_2 = 0.0012$; bootstrap confidence interval (CI) = 0.0010–0.0014; details in *SI Materials and Methods* and *SI Results*] as well as in all trait-specific data subsets (Fig. S3*C*), indicating the data meet the requirements to detect inbreeding depression, if any is present (9).

Juvenile Traits. We found that increased F_{grm}, but not F_{ped}, was associated with significantly lower birth weight (model M2 in Table 1; sample sizes for all traits are given in Table S2, and estimates for all fitted fixed and random effects are given in Table S3). Estimated effect sizes were highly similar when different ancestry information thresholds were used for F_{ped}, or when using alternative estimators of genomic inbreeding instead of F_{grm} (Fig. S4). Inbred calves with $F_{\mathrm{grm}} = 0.125$ (i.e., a value that might result from a half-sib mating, and which is the 99th percentile among newborns in this dataset) were 0.28 ± 0.09 kg lighter ($-\beta \pm$ SE) than outbred calves (taken as $F_{\mathrm{grm}} = 0$). This is of similar magnitude to the difference in average birth weight between the sexes (0.33 ± 0.04 kg, model M2 in Table S3), and constitutes a 4.4% decrease relative to the average birth weight of 6.39 kg. Interactions between sex and F_{ped} or F_{grm} on birth weight were nonsignificant ($P > 0.1$).

As previously reported (e.g., refs. 22 and 26), lower birth weight was associated with reduced survival of neonates, of calves during the first winter of life, and of yearlings (models M3–M5 in Table S3). Over and above the effect of birth weight, inbreeding coefficients (both F_{ped} and F_{grm}) were negatively associated with calf survival over the first winter and with yearling survival. There is a similar negative effect of later birth date on survival during each of these periods (models M3–M5 in Table S3), but no association was found between a calf's date of birth and its inbreeding

Table 1. Estimated effect sizes for pedigree and genomic inbreeding coefficients (F_{ped} and F_{grm}) in a range of fitness correlated traits

Model		Focal's F_{ped}			Mother's F_{ped}			Focal's F_{grm}			Mother's F_{grm}		
No.	Trait	β	SE	P	β	SE	P	β	SE	P	β	SE	P
							Juvenile traits						
M1	Birth date*	2.49	(13.4)	0.85	−15.0	(19.9)	0.45	−16.3	(10.4)	0.12	−17.9	(14.2)	0.21
M2	Birth weight	−1.29	(0.86)	0.14	−0.58	(2.12)	0.78	**−2.25**	**(0.69)**	**0.001**	−1.95	(1.46)	0.18
M3	Summer survival†	1.12	(3.11)	0.72	−3.47	(4.18)	0.41	0.11	(2.63)	0.97	−4.93	(3.21)	0.12
M4	Winter survival†	**−17.3**	**(3.55)**	**<0.001**	−4.65	(4.68)	0.32	**−12.4**	**(2.53)**	**<0.001**	−3.06	(3.29)	0.35
M5	Age 1–2 y survival†	**−9.82**	**(4.94)**	**0.05**	−5.56	(5.39)	0.30	**−12.3**	**(3.60)**	**<0.001**	−7.99	(4.24)	0.06
							Combined juvenile traits						
M6	Age 0–2 y survival	**−13.4**	**(3.31)**	**<0.001**	−5.02	(4.48)	0.26	**−13.5**	**(2.46)**	**<0.001**	**−8.71**	**(3.30)**	**0.006**
							Adult traits						
M7	Female AFR*	−1.96	(3.51)	0.56	0.56	(2.70)	0.86	−2.14	(1.97)	0.28	3.46	(2.04)	0.09
M8	Female annual survival	−2.07	(4.97)	0.65	NF			−6.84	(3.46)	0.05	NF		
M9	Male annual survival	−8.41	(4.66)	0.08	NF			−1.19	(3.80)	0.74	NF		
M10	Female ABS	−6.24	(4.32)	0.15	NF			**−8.35**	**(3.22)**	**0.008**	NF		
M11	Male ABS	−11.9	(7.37)	0.08	NF			**−13.0**	**(3.43)**	**<0.001**	NF		

Estimates for all fitted fixed and random effects in each model are given in Table S3. For the juvenile traits (M1–M6), the sexes were analyzed together, and either F_{ped} or F_{grm} of both focal individual and mother were fitted in the same model. Annual survival (age 2+ y) and ABS (age 5+ y) were analyzed for each sex separately, and AFR was analyzed for females only. Significant effects ($P \leq 0.05$) are indicated in bold. Sample sizes ranged from 232 to 1783 (see Table S2). NF, not fitted.

*Multiplied by −1, as larger values are associated with decreased fitness.

†Birth date and birth weight fitted as covariates.

coefficient (M1 in Table 1). Overall, these results show that inbreeding depression in juvenile survival is channeled both via decreased birth weight and, as the calf develops, via other mechanisms that affect survival.

The combined effects of a juvenile's inbreeding coefficient on its birth weight (M2) and survival during the three juvenile periods (M3–M5) resulted in a large decrease in the probability of surviving to independence at age 2 y (Table 1, model M6). As an example, an inbred female calf ($F_{grm} = 0.125$) showed a 44% decrease (95% CI: 26–60%) in predicted survival probability compared with a female calf of $F_{grm} = 0$, and a similarly inbred male calf was 49% (30–66%) less likely to survive (Fig. 2*A*, depicting average over both sexes).

Effect of the Mother's Inbreeding Coefficient on Offspring Traits. Increased maternal F_{grm} was strongly associated with a decrease in offspring survival from birth to age 2 y (Fig. 2*B*, model M6 in Table 1), whereas for maternal F_{ped} the effect was much smaller and nonsignificant. The effect of maternal inbreeding coefficient on offspring fitness has an effect size amounting to two-thirds that of offspring F_{grm} (M6). This effect appears diffuse but cumulative in nature: There was no significant effect of maternal inbreeding on parturition date, offspring birth weight, or offspring survival through any of the subperiods to age 2 y, although all coefficients were negative (models M1–M5).

Adult Traits. There was no association between a female's own inbreeding coefficient and her age at first reproduction (M7, typically age 3–5 y). Daughters of more inbred mothers tended to have earlier age at first reproduction (AFR), although this was not statistically significant ($P = 0.087$) (see also *SI Discussion*).

There was no significant association between annual survival after age 2 y and F_{ped} or F_{grm}, in either females (M8) or males (M9), although we did observe a decrease in both average F_{grm} and average F_{ped} with age for both sexes (Fig. S5*A*), and survival tended to be lower in females with higher F_{grm}, and in males with higher F_{ped} (M8 + M9, respectively).

Among both females and males aged 5 y and over, we found that increased F_{grm} was associated with decreased ABS (models M10 and M11 in Table 1 and Table S3). There was no significant association between F_{ped} and ABS in either sex.

Lifetime Fitness. The negative association between inbreeding coefficients and each of the aforementioned traits should contribute to inbreeding depression in LBS (the number of offspring produced) and, for females, LRS (the number of offspring surviving

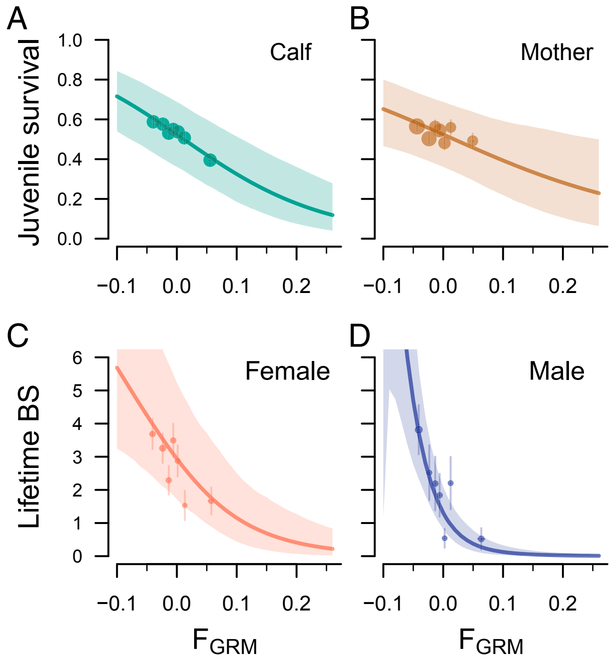

Fig. 2. The relationship between F_{grm} and juvenile survival and LBS. Survival from birth to age 2 y decreases with the genomic inbreeding coefficient F_{grm} of the offspring (*A*), as well as with F_{grm} of the mother (*B*), and LBS decreases with F_{grm} in both females (*C*) and males (*D*). Points show observations, grouped into seven bins using the septiles among newborns (−0.029, −0.018, −0.010, −0.002, 0.007, and 0.020), with point sizes proportional to number of observations in each bin, and error bars indicating 1 SE around the mean. Lines show the fitted models, and shaded areas show the 95% CI; estimated slopes and SEs are given in Table 1. F_{grm} ranged from −0.10 to 0.36 among neonates, and from −0.10 to 0.19 among adults.

Table 2. Estimated effects of genomic inbreeding coefficients F_{grm} and F_{ped} on LBS (number of calves produced) in each sex, and on LRS (number of calves surviving to independence at age 2 y) in females

Model		F_{ped}						F_{grm}					
		Prob. LBS* > 0			LBS* when >0			Prob. LBS* > 0			LBS* when >0		
No.	Trait	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
M12	Female LBS	**−13.7**	**(6.20)**	**0.02**	−0.08	(1.86)	0.98	**−16.8**	**(4.69)**	**<0.001**	−2.12	(1.44)	0.13
M13	Female LRS	**−14.2**	**(6.77)**	**0.03**	**−9.59**	**(3.78)**	**0.003**	**−18.7**	**(5.62)**	**<0.001**	**−4.13**	**(2.07)**	**0.05**
M14	Male LBS	**−31.7**	**(11.8)**	**<0.001**	−23.3	(13.0)	0.06	**−18.6**	**(5.25)**	**0.001**	**−14.9**	**(3.92)**	**<0.001**

The models simultaneously estimate the effect of inbreeding coefficients on the probability of breeding ("Prob. LBS > 0," binomial part) and on the number of offspring, conditional on having at least one offspring ("LBS when > 0," truncated Poisson part). Significant effects ($P \leq 0.05$) are indicated in bold. The estimates for the random effects in each model are given in Table S3. Sample sizes ranged from 384 to 458 (see Table S2).

*LRS, rather than LBS, is used for M13.

to 2 y). To estimate total inbreeding depression, we fitted a hurdle Poisson model, which accounts for the excess of zeros in the distributions of LBS and LRS (details in *SI Materials and Methods*). The probability of having at least one offspring (and so passing the hurdle) decreased strongly with increasing inbreeding coefficient in both sexes, for both F_{ped} and F_{grm} ("Prob. LBS > 0" in Table 2, "hurdle" in Table S3).

In females, there was no further effect of inbreeding on LBS, the number of offspring born (M12, "when > 0" in Table 2, "Poisson" in Table S3). However, there was an effect on LRS, the number of offspring that survived to independence at age 2 y (M13), in line with the effect of maternal inbreeding coefficient on offspring survival (M6). Similarly, among males that sired at least one offspring (LBS > 0), those with a higher F_{grm} sired significantly fewer offspring (M14). There was no significant association with F_{ped} (Table S3).

Combining the effects of inbreeding on the hurdle and on the truncated Poisson distribution, inbred females with $F_{\mathrm{grm}} = 0.125$ had a predicted 72% reduction in LBS, compared with an average female with $F_{\mathrm{grm}} = 0$ (95% credibility region: 43–88% reduction) (Fig. 2*C*), and a 79% reduction in LRS (52–92%). Males with $F_{\mathrm{grm}} = 0.125$ had a predicted 95% (86–98%) reduction in LBS (Fig. 2*D*). Note that, for both sexes, individuals with $F_{\mathrm{grm}} < 0$ had a higher LBS than those with $F_{\mathrm{grm}} = 0$ (Fig. 2 *C* and *D*), making $F_{\mathrm{grm}} = 0$ a conservative benchmark to use as "outbred" individuals.

Discussion

The substantial inbreeding depression in lifetime fitness found here is partly due to inbreeding depression in juvenile survival, which has been reported in previous studies of this population (20, 22) and in many other species (1). In addition, we found that increased genomic inbreeding coefficients were associated with decreased ABS in adults of both sexes. Moreover, increased maternal inbreeding coefficients decreased offspring survival to independence. In contrast, when using pedigree-derived inbreeding coefficients, we could detect significant inbreeding depression in juvenile survival but not in any of the adult traits.

Variance in Inbreeding and Identity Disequilibrium. The Rum red deer study population was expected to have high variance in inbreeding coefficients, due to a limited population size, recent admixture with red deer from the mainland (21), ongoing admixture with red deer from other parts of the island, a strongly polygynous mating system, and higher levels of consanguineous matings than expected under random mating (27). Despite this, the variance in inbreeding appears relatively low compared with other pedigreed vertebrate populations, at $\mathrm{var}(F_{\mathrm{grm}}) = 0.0011$ and $\mathrm{var}(F_{\mathrm{ped}}) = 0.0008$ among neonates (Fig. S5*B*), compared with $\mathrm{var}(F_{\mathrm{ped}}) = 0.0004$–$0.0192$ (median 0.0031) among 18 mammal and bird species listed by Grueber et al. (28). However, this list includes various populations with a very small number of founders; data on the typical variance of inbreeding in animal populations is currently lacking.

Variance in inbreeding coefficients typically decreases with age, due to the selective disappearance of the most inbred individuals (see Fig. S5*B*), resulting in a lower identity disequilibrium (g_2) among adults. Here, compared with neonates, g_2 was 33% lower among adult females and 21% lower among adult males (including immigrant males; see also Fig. S3*C* and *SI Discussion*). Consequently, estimating inbreeding depression in adult traits is even more challenging than in juveniles, and more markers are required to estimate genome-wide homozygosity accurately. The large number of markers used here ensured that g_2 was significantly different from zero for all data subsets (Fig. S3*C*), although, across traits, there was a (nonsignificant) positive trend between g_2 and estimated inbreeding depression (Fig. S3*D*).

Genomic Versus Pedigree-Based Estimates. More F_{grm}- than F_{ped}-based estimates of inbreeding depression differed significantly from zero; the main reason for this was that the SEs when using F_{grm} were consistently smaller than when using F_{ped} (Table 1), in line with expectations based on other studies (14, 16). For most (but not all) traits, we found that F_{grm} additionally resulted in slightly larger point estimates, but none of the differences between the estimates were significant (Table 1 and Fig. S6*A*). Exceptions to the general pattern of larger estimates for F_{grm} may thus be due to chance alone.

The difference in performance between F_{ped} and F_{grm} can be attributed to a number of factors. First, there was a difference in the available sample size (Table S2). However, even, when using a smaller data set including only individuals for whom both metrics were known, the narrower confidence intervals for F_{grm} compared with F_{ped} remained (Fig. S6 *B*–*D*). Second, although F_{ped} was based on a standardized minimum amount of pedigree information, this metric will contain error due to missed inbreeding in the highly variable depth of pedigree available for different individuals. Third, as described in the Introduction, F_{grm} captures variation in IBD around the pedigree expectation. Note that the pattern is unlikely to have been caused by the somewhat different scales of the two estimators because, all else being equal, the wider range of observed values for F_{grm} (−0.10–0.36) than for F_{ped} (0–0.27) would result in shallower slopes of trait values against F_{grm} than against F_{ped}.

The difference between pedigree and genomic estimates of inbreeding depression reported here is much more pronounced than in a similar comparison focusing on the estimation of heritability and genetic covariances in a study population of Soay sheep on St Kilda, NW Scotland (29). One reason for this is that individuals who lack some ancestry information may have a highly imprecise F_{ped}, while having accurate pedigree relationships with their many (half-)siblings, descendants, and known ancestors. All these pairwise relationships contribute to the estimate of heritability, whereas only the relatively small number of direct ancestors contribute to F_{ped}.

Inbreeding Depression in Fitness Components. Generally, inbreeding depression is expected to be stronger in traits more closely associated with fitness (30). Our findings were in line with this expectation, with, for example, individuals with $F_{\text{grm}} = 0.125$ showing only a small reduction in birth weight (−4.4%) compared with the reduction in juvenile survival (−47%), and an even larger reduction in LBS (−72% to −95%).

This trend was not followed by the two adult fitness components considered. Previous studies in this red deer population showed that female LBS is more strongly determined by longevity than by ABS, whereas male LBS is about equally determined by both components (19, 31). This is a consequence of the breeding biology of red deer, with a maximum of one calf per year for females, and hence a greater advantage of longevity over ABS compared with males. Based on this, one would expect females to show stronger inbreeding depression in survival than in ABS, and that estimates for both traits would be similar in males. In contrast, in inbred females (again using $F_{\text{grm}} = 0.125$), we found a stronger reduction in ABS (−24%) than in adult survival (−4%), and the difference was even larger for males (ABS −79%, survival −1%). The reason for this unforeseen pattern is not clear, and seems unrelated to sample size (Table S2) or identity disequilibrium (Fig. S3*C*). The relative magnitudes of inbreeding depression in these traits are likely to be species-specific, but, interestingly, in song sparrows, a similar pattern was found, with a larger effect of inbreeding coefficients on male than female annual reproductive success (ARS), and no detectable effect on adult survival (10). Similarly, in Darwin's finches, inbreeding depression in the annual probability of breeding was more consistent than in annual survival (32).

Inbred Mothers. An effect of the mother's inbreeding coefficient on offspring survival is rarely reported, but possibly contributed to the documented reduced ARS in song sparrows (10) and bottlenose dolphins (33). In the deer, offspring of inbred females ($F_{\text{grm}} = 0.125$) had a 31% lower probability of survival to independence, which, together with the reduced ABS, resulted in a 47% decrease in ARS for inbred females (assuming independent effects on ABS and offspring survival). The relative magnitudes of inbreeding depression in ABS and offspring survival, and the most affected offspring age class, are likely to vary between species. For example, an earlier study in the song sparrows documented a negative effect of female F_{ped} on decreased hatching rates, but not on egg number or subsequent fledging rates (8), whereas, in wandering albatross, microsatellite homozygosity was associated with offspring fledging rates, but not number of hatched offspring (34).

On Rum, red deer females do not conceive in years when they are in poor condition at the rut, and this may provide an opportunity for compensation in inbred females. By foregoing the cost of reproduction on survival (M8) and subsequent fecundity (M10), the net effect of a female's inbreeding coefficient on LBS is diminished, and so was undetectable among those who bred at least once (M12). Similar discrepancies between effects on annual or seasonal and lifetime measures of fitness due to compensation can be found, for example, when inbreeding affects brood success in birds, but inbred females increase the number of breeding attempts (35). These results emphasize the importance of considering the entire life cycle, including effects of parental inbreeding coefficients on juvenile survival, when estimating the total effect of inbreeding depression.

Inbreeding Depression in Fitness. We found inbreeding depression both in whether or not a male sired any offspring, and, if he did, in how many offspring he sired during his lifetime (model M14). In contrast, in females, although there was inbreeding depression in whether or not a female bred, if a female did breed, there was no association between inbreeding coefficient and her LBS (M12), but there was a negative association with her lifetime number of recruits (LRS, M13). These results for LBS are consistent with a previous heterozygosity fitness correlation of LBS using microsatellites, in which the slope was steeper for males than for females (25). The difference between the sexes is probably partly a consequence of the polygynous breeding system of red deer with strong male–male competition, which leads to higher variance in LBS in males than in females. In song sparrows, which are somewhat polygynous (but much less so than red deer), the magnitude of inbreeding depression in LRS was twice as large in males as in females (10). However, in general, this hypothesis is difficult to verify, as few other vertebrate studies have documented the effect of inbreeding coefficients on LBS or LRS in both sexes.

Materials and Methods

Study Population. The study area is located in the North Block of the Isle of Rum off the west coast of Scotland, and contains about one-quarter of the adult red deer on the island. Red deer are highly seasonal, polygynous breeders, and, during the autumn rut, adult males defend groups of females against competitors for the chance to mate. Deer are free to move in and out of the study area, and are unmanaged within it, but are subject to annual culls on the remainder of the island. For further details, see refs. 18 and 22. The research was conducted following approval of the University of Edinburgh's Animal Welfare and Ethical Review Body and under appropriate UK Home Office licenses.

We considered all individuals born between 1981 (when the population reached carrying capacity following cessation of culling in 1973) and 2013 for which sufficient life history information was available (see *SI Materials and Methods* for details). Sample sizes for each trait are given in Table S2. Data are available on FigShare (10.6084/m9.figshare.2075584.v1).

Phenotypic Traits.

Birth weight. Most calves are caught within a few days of birth, to take measurements and apply artificial markings. In the models of birth weight, capture weight was used as a response variable, and age at capture (in hours) was fitted as a covariate. When birth weight was used as a covariate, it was estimated from a linear regression of body mass on age at capture (slope: 0.01696 kg/h).

Survival. Regular censuses throughout the year and mortality searches during winter provide accurate information on death date for most individuals. Individuals who were shot, emigrated, or with unknown fate were excluded from survival analysis in that particular year only. Annual survival was evaluated between 1 May and 1 May, rather than in calendar years, as most mortality occurs in winter.

Breeding success. Females give birth to, at most, a single offspring per year. The regular censuses, plus intensive observations in the calving season, provide pregnancy statuses and parturition dates for all resident females, as well as AFR, typically between 3 y and 5 y. For ABS, all individuals age 5 y and over seen in censuses during the rut (males) or calving season (females) in a given year were included in the data. This lower age limit avoided confounding ABS with AFR in the females, and enabled comparison among potentially reproducing mature males only (0.7% of individuals were sired by males aged 2–4 y).

LBS. LBS (birth–death) was calculated for all individuals in the cohorts 1981–2000 who had either died a natural death or were still alive in 2013 ($n = 4$ males) or 2015 ($n = 9$ females). This approach, following ref. 23, minimizes bias toward individuals who died young, whereas the few individuals still alive had obtained almost all of their LBS: 1.9% of pregnancies were after age 15 y, and 2.5% of calves were sired by males over age 13 y (we know the mothers but not fathers of calves born in 2014 and 2015). For LRS (total number of offspring who survived to age 2 y, birth–death of focal individual), we considered the cohorts 1981–1998, otherwise using the same criteria as for LBS.

DNA Extraction and SNP Data. DNA was extracted from neonatal ear punches, postmortem tissue, and cast antlers (details in *SI Materials and Methods*). Genotyping was performed using the cervine Illumina BeadChip, and quality control was done in Genome Studio (Illumina) and PLINK (36) (SNP call rate > 0.99, individual call rate > 0.9, minor allele frequency > 0.01; further details in *SI Results* and Fig. S1). In total, 2,254 individuals were genotyped at 37,410 polymorphic SNPs. The number of markers did not limit the precision of our estimates (*SI Materials and Methods*, *SI Results*, and Fig. S3*B*).

Inbreeding Coefficients. The existing pedigree (37) was extended and improved, using the better resolution of the SNP data compared with the previously used microsatellite markers, and the additional individuals genotyped for the SNPs. A likelihood-based pedigree reconstruction method was developed that identified parents as well as second-degree relatives, using 440 SNPs (details in *SI Materials and Methods*). F_{ped} was calculated in the R-package Pedantics (38) using Wright's path approach (39).

The genomic inbreeding estimator used [$\hat{F}^{\text{III}}$ in Yang et al. (25)] estimates the correlation between uniting gametes, Wright's original definition of the inbreeding coefficient (39). It is highly correlated to average marker homozygosity, and to an estimator that corrects for expected homozygosity (36) (details in *SI Materials and Methods* and Figs. S4 and S2) but has a lower sampling variance (25) (Fig. S3).

Statistical Analysis. Detailed statistical analyses for many traits in the study population have been published before (18, 19), and effects of multiple explanatory variables other than inbreeding on the focal traits are well known. To maximize sample sizes, we included all individuals with either F_{ped} or F_{grm} known, rather than using a smaller, identical set of individuals with both metrics known. For each trait, all known fixed and random covariates were fitted in addition to the inbreeding coefficient(s), and we attempted to use the same covariates for each of the juvenile traits and each of the adult traits, where appropriate (see Table S3). All analysis was done in R (40), using ASREML-R for the normally distributed traits, LME4 for the juvenile survival traits, and MCMCglmm (41) for the remaining traits. To ensure convergence of models in LME4, fixed effects were standardized. Details of the statistical analysis including covariates are given in *SI Materials and Methods*.

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