The social environment has little impact on inbreeding depression in a social mammal

King To Chan[1,2], Alexandre Courtiol[3], Leonie F. Walter[3,4,5], Oliver P. Höner[6,7], Larissa S. Arantes[3,8], Eve Davidian[7,9], Philemon Naman[7], Loeske E.B. Kruuk[1], Josephine M. Pemberton[1], Kasha Strickland[1*]

1. Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh, UK
2. The Roslin Institute, Easter Bush Campus, University of Edinburgh, UK
3. Department of Evolutionary Genetics, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
4. Department of Ecological Dynamics, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
5. Gjærevoll Center for Biodiversity Foresight Analyses, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
6. Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
7. Ngorongoro Hyena Project, Ngorongoro Conservation Area, Tanzania
8. Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Berlin, Germany
9. Evolutionary Anthropology Team, Institute of Evolutionary Sciences of Montpellier (ISEM), University of Montpellier, CNRS, IRD, Montpellier, France

* author to whom all correspondence should be addressed

email: kasha.strickland@ed.ac.uk

Abstract

Inbreeding depression describes the decline in fitness caused by breeding between relatives and is now known to be widespread in natural populations. Yet, its relative strength across different fitness components and its sensitivity to social and demographic environments are poorly understood. Using nearly 30 years of life-history, behavioural, pedigree and genomic data from a wild population of spotted hyenas (*Crocuta crocuta*) in the Ngorongoro Crater, Tanzania, we tested for inbreeding depression in three key fitness components and evaluated whether it is moderated by socio-demographic factors. We estimated individual inbreeding using both pedigree-based and genomic-based approaches. We found evidence for inbreeding depression in two out of three fitness components: when inbreeding coefficients increased, both lifespan and lifetime reproductive success decreased. Juvenile survival also decreased, but not significantly so. We found little evidence that the strength of inbreeding depression varied systematically with sex, social rank, or clan size. Genomic and pedigree estimates of inbreeding yielded broadly comparable conclusions about inbreeding depression when the pedigree was restricted to individuals with at least three known grandparents. Together, these results demonstrate inbreeding depression may be present, and yet not strongly influenced by the social environment, in a socially structured wild population.

Keywords: F_{GRM}, F_{PED}, inbreeding depression, LRS, relatedness, social mammals, spotted hyena.

Introduction

Inbreeding depression refers to a decline in fitness caused by increased homozygosity that results from inbreeding. When related individuals reproduce, their offspring are more likely to inherit alleles that are identical-by-descent (IBD) [1,2]. Inbreeding depression then arises through the expression of partially recessive deleterious alleles or the loss of heterozygote advantage [3,4]. Because of its effects on fitness, inbreeding depression can influence evolutionary trajectories and population viability [5]. Moreover, it poses particular risks for small populations [6,7] and can hinder adaptation to environmental change [8]. An understanding of inbreeding depression is thus essential for effective conservation or management of wild populations [9].

Inbreeding depression has been found across taxa in both laboratory and wild populations [10–12], and is increasingly recognised to be dependent on the environment [13,14]. In particular, exposure to stressful environments can exacerbate the fitness costs of inbreeding [15,16]. The social environment, defined by an individual's interactions and associations with conspecifics, represents a pervasive and particularly relevant component of this environmental context [17]. In particular, the potential for inbreeding increases with the kin structure of the population, which is high in many wild species [18–20]. However, social interactions and group living may also mitigate inbreeding depression by buffering individuals against harsh environmental conditions [21], unless the local density, and the resulting intraspecific competition, are high [22]. Moreover, in species structured by social hierarchies, rank-related differences in fitness, caused by differential access to resources within social groups, may further modulate the strength of inbreeding depression within social groups. Understanding how variation in socio-demography moderates the strength of inbreeding depression is therefore crucial to explaining and predicting variation in its strength across natural populations.

Historically, inbreeding coefficients have been estimated using pedigree-based methods (FPED) [2,23]. However, FPED does not directly estimate the realised genomic consequences of inbreeding and is limited by the quality of pedigrees, which may be shallow or incomplete in wild populations [24,25]. This may lead to systematically biased estimates of inbreeding and poor accuracy in predicting the genomic outcomes of inbreeding [26–28]. The increasing affordability and availability of genomic data has facilitated a shift to using molecular estimates, which more directly capture realized homozygosity [26,29]. The current gold standard of genomic inbreeding estimators is the detection of runs of homozygosity (ROH), which are considered proxies for IBD segments [30]. However, whilst estimates of ROH offer good accuracy in capturing whole-genome homozygosity and are compatible with a wide range of genotype-by-sequencing methods [31], they require chromosome-level reference genomes or linkage maps for the genotyped SNPs. In species without such resources, genomic relationship matrix-based estimates (FGRM) have proven to be robust alternatives [31,32]. These use single nucleotide polymorphism (SNP) data to quantify individual homozygosity relative to population allele frequencies [33] and have been validated for detecting inbreeding depression in both simulations and captive populations [24,31]. Nonetheless, because many long-term field studies still rely on pedigrees, comparing FPED and genomic estimates remains essential to understand the strengths and limitations of each approach [34,35].

In this study, we capitalised on an exceptionally rich dataset of nearly three decades of data to test for inbreeding depression and to examine how socio-demographic factors influence its strength in a population of spotted hyenas (*Crocuta crocuta*) in the Ngorongoro Crater, Tanzania. Spotted hyenas provide an excellent system to explore how inbreeding depression interacts with the social environment. They live in social groups, called clans [36,37], within which philopatric females form a linear dominance hierarchy, and both social rank and social associations are maternally inherited [38,39]. As a result, hyena clans are strongly kin-structured [36,38,40], increasing the risk of inbreeding in this polygynandrous species. Yet, most males disperse and start their reproductive life in a non-natal clan, and females preferentially mate with non-natal males, which are behaviours that could limit inbreeding [41–43]. In addition, the strong links between socio-demography and fitness in hyenas provide an opportunity to test whether the social environment moderates the strength of inbreeding depression. For instance, fitness variation in hyenas is strongly shaped by social rank [38,41,44,45], which influences access to food and levels of competition and may therefore mediate the fitness consequences of inbreeding. Moreover, clans vary in size (and therefore population density), social structure, kinship composition, and the habitats they occupy [46,47], suggesting that the severity of inbreeding depression may also differ among social groups.

We used nearly 30 years of fitness and life-history data collected on 3265 individuals from eight clans, with a nine generation pedigree and a panel of ~ 22K SNPs to (1) describe the extent of inbreeding in the population, (2) combine empirical life-history data with inbreeding measures to estimate the strength of inbreeding depression, (3) test whether inbreeding depression varies between sexes, between clans or according to social rank and clan size, and (4) assess if F_{GRM} and F_{PED} differ in their performance in estimating inbreeding depression.

Methods

Study population and pedigree

The spotted hyena population inhabiting the Ngorongoro Crater in Tanzania has been studied since 12th April 1996. The Ngorongoro Crater is a 300 km^2 large, intact caldera that is part of the larger Serengeti-Mara ecosystem. Here, we use data collected between the start of the project and 1st September 2025. During this period, the population size varied between 165 and 609 individuals which were distributed among eight social clans with average sizes ranging between 26 and 73 members. Individuals were identified based on their unique spot patterns, scars, ear notches and other visual cues. Estimates of life history events, such as dates of birth, deaths and dispersal were determined based on sightings recorded during near-daily visits to the Crater and behavioural observations [for details, see 41]. Individuals were sexed visually through binoculars by the shape of the phallic glans when erected. DNA samples were collected as hair roots, faecal epithelium cells and other tissue for 2181 individuals. Social ranks for individuals in the population were determined from a sociometric matrix for each clan based on dyadic agonistic interactions, as well as maternal rank inheritance and social queueing for new members [48]. Females reproduce year-round, typically producing 1–2 cubs per litter (1891 out of 1901 litters, or 99.5%), predominantly single-sired (415 out of 489, or 84.9% of non-singleton litters with full paternity information), that nurse for ~ 13 months. Parentage was determined using a combination of observations of nursing behaviours and genetic parentage assignments based on analyses of nine polymorphic microsatellite markers [49,50].

As of September 2025, the full pedigree included a total of 3137 individuals, including 3012 born in one of the eight Crater clans, 32 males of uncertain origin found in such clans at the start of the study period, and 93 individuals born outside the Crater but sexually active in the Crater. This pedigree spans a maximum of nine generations (mean depth per individual born in one of the eight clans and alive as of 1st September 2025 = 5.36 generations), including 1843 individuals with both parents identified, 964 with a known mother only, and 4 with a known father only. Amongst the 326 hyenas with no known ancestors, 134 were alive at the start of the study ("founders").

SNP genotyping

Genotype-by-sequencing data were generated as described in Arantes et al. [51]. Using the collected samples described above, DNA was extracted using commercially available toolkits. The 3RADseq method was used to construct the sequencing libraries using EcoRl, Xbal and Nhel restriction enzymes, overcoming the degradation and inconsistency in the quality of non-invasive DNA samples [52,53]. The resulting products were pooled and cleaned using 0.8X CleanPCR magnetic beads (GC biotech). To prevent amplification bias toward small DNA fragments, fragment size selection was performed with the BluePippin software to obtain samples with fragment sizes between 390 and 450 bp, followed by PCR reactions to construct the final sequencing libraries, which were screened by the Agilent Tapestation system on a MiSeq platform (Illumina).

The base reads in the final sequencing libraries were then submitted to an automated pipeline to perform preprocessing quality control and produce spike-in reads [51]. These spike-in reads were then mapped to the *Crocuta crocuta* genome using *Bowtie2* [54]. Individuals with less than 50% mapped reads were removed. DNA sequences were re-pooled giving more weight to individuals with fewer reads to obtain more balanced libraries. Re-pooled libraries were constructed and rechecked following the procedures described before being submitted for sequencing using two lanes of the NovaSeq S4 platform (Illumina). NovaSeq reads were pre-processed using the automated pipeline described above and had their adapters trimmed using *Cutadapt* [55]. The resulting reads were mapped to the *Crocuta crocuta* genome using *Bowtie2*. The software *RADSex* was used to identify and remove local markers that fell on a sex chromosome [56]. Then, SNPs were discovered and genotyped using the *Stacks* referencing pipeline v2.61 [57], keeping one SNP per RAD locus to reduce linkage between markers. Only loci genotyped in at least 60% of individuals were retained. The reads for individuals with greater than 2.5 million reads were subsampled to even up the coverage per site among individuals. The resulting SNP calls were further filtered using thresholds with a minimum sequencing depth of 10 and a maximum sequencing depth of 110 [51]. As a result, a total of 1181 spotted hyenas were genotyped at a panel of 69816 SNP markers.

As Mendelian errors (ME) reflect possible genotyping errors, SNPs showing frequent ME should be removed to improve genotype accuracy. ME were calculated as the proportion of opposite homozygotes in parent-offspring pairs from the existing pedigree using the R package *Sequoia* [58]. SNPs were removed if they had an ME rate of more than 1% (N = 3241 SNPs). We then removed samples if they had more than 50% missing genotype calls (13 individuals discarded).

Individual inbreeding coefficients

The pedigree-based inbreeding coefficients (F_{PED}) were calculated for individuals from the existing pedigree using the *ggroups* R package [59,60]. This approach provides a genome-wide prediction of IBD over multiple generations.

In the absence of an chromosome-level reference genome, we used the inbreeding coefficient $\hat{F}^{\mathrm{III}}$ as our genomic measure, estimated in *PLINK* 1.9 [33,61]. Based on the correlation between uniting gametes, $\hat{F}^{\mathrm{III}}$ estimates the individual weighted average of homozygosity across SNPs, giving more weight to minor alleles using equation 1:

Eq 1. $\hat{F}^{\mathrm{III}} = \frac{1}{m} \sum_{j}^{m} \frac{X_j^2 - \left(1+2p_j\right)X_j + 2p_j^2}{2p_j\left(1-p_j\right)}$

where m is the number of SNP markers for individual j, X is the observed number of the reference allele (0, 1 or 2), and p is the estimated population-wide allele frequency of the reference allele [33]. We hereafter refer to this estimate as F_{GRM}.

Prior to estimating F_{GRM}, we removed individuals that were born outside of the Crater as this may bias the estimation of the population's allele frequencies. Additionally, we removed SNPs with a minor allele frequency (MAF) of less than 1%, and a marker genotype missingness of more than 20%. As a result, F_{GRM} was estimated for 1119 individuals using a final panel of 21929 SNPs. To ensure SNP filtering did not affect F_{GRM} estimates, we evaluated the impact of SNP filtering parameters on estimates of F_{GRM} by applying a range of thresholds for marker genotype missingness and MAF. Missingness or MAF thresholds above 1% did not have a significant impact on F_{GRM} estimates (Table S1 and S2). Finally, we removed two individuals that had very low F_{GRM} values ($F_{GRM} < -0.1$), which may reflect a higher genotyping error in those samples. All filtering of the SNP dataset was done in *PLINK* 1.9 [62].

Measures of fitness

To investigate inbreeding depression, we assessed three key measures of fitness and life-history: juvenile survival, lifespan, and lifetime reproductive success (LRS). Juvenile survival was measured as a binary variable describing whether or not an individual survived to adulthood. Individuals were considered as adults from two years of age onwards as this is the age by which high-ranking females typically start reproducing and males have viable sperm in their testes [63,64]. Analyses using a 1-year cut-off for juvenile survival yielded similar results and inferences. Individuals' lifespans were calculated as the length in years from their date of birth to their date of death. LRS was defined as the total number of offspring produced by an individual throughout their lifetime.

Data selection

For analyses of inbreeding depression on juvenile survival, we used a dataset that included individuals that were born into one of the eight main Crater clans after the first observation in the dataset but before 1st September 2021, without restriction as to when they died (N = 2526). This ensured that we included individuals in analyses that were seen regularly enough to reliably assess the survival status at two years of age.

For analyses of inbreeding depression on lifetime fitness (lifespan and LRS), we used a subset of the dataset that included individuals that were born into one of the eight main Crater clans after the first observation in the dataset but before 1st September 2015, and had died before the last observation in the dataset (N = 1748). This ensured that we (1) had a set of individuals for which we had complete fitness and life history measures, and (2) did not generate biases in our analyses by including individuals born into cohorts where many of the individuals were still alive by the end of the study period. We identified 1st September 2015, i.e., ten years before the last observation, as the right-censor date by identifying it as the year in which approximately 90% of the cohort had died by the end of the study period. We also conducted sensitivity analyses using alternative thresholds of 1st September 2013 (N = 1544) and 1st September 2017 (N = 2013), but these did not change the qualitative inferences presented in the results section (see supplementary information).

In both the juvenile survival and lifetime datasets', we removed individuals that were adopted, or for which the adoption status was uncertain (i.e., social mother unknown), to ensure accurate modelling of maternal effects (see *statistical analyses*), resulting in the loss of 137 individuals for the dataset used for the study of juvenile survival, and 83 individuals for the one used to study lifespan or LRS. We also discarded individuals that did not have complete data for all fixed and random effects included in the models (see *statistical analyses* for predictors).

For analyses of inbreeding depression using F_{GRM}, which retained only SNP-genotyped individuals, the dataset for juvenile survival included a total of 993 individuals (N = 496 males and 497 females, proportion of juveniles surviving to 2 years = 0.69) and for the one used for lifetime fitness models included a total of 768 individuals (N = 379 males and 389 females, mean LRS = 2.96, mean lifespan = 6.39 years). For analyses of inbreeding depression using F_{PED}, we kept individuals for which both parents and at least three grandparents were known. This filtering step was conducted to ensure estimates of inbreeding from the pedigree were not biased too much downward by missing ancestry, whilst preventing excessive data loss (e.g., when removing individuals that had any unknown grandparents). This resulted in a dataset of 1254 individuals for juvenile survival (N = 632 males and 622 females, proportion of juveniles surviving to 2 years = 0.63) and 793 individuals for lifetime fitness (N = 377 males and 416 females, mean LRS = 2.07, lifespan = 5.23 years).

Statistical analyses

We tested for evidence of inbreeding depression in juvenile survival, lifespan, and LRS using linear mixed-effects models in R statistical environment [65] using the R package *spaMM* [66]. We fitted one model per measure of fitness: juvenile survival was modelled as a logistic regression using a generalised linear mixed-effects model (GLMM) with the binomial family and a logit link function; lifespan (in years) was normalised via a scaled rank transformation and then modelled in a linear mixed-effects model (i.e., Gaussian family and identity link function). More specifically, we extracted the ranks of individual lifespans using the R function RANK and divided these ranks by N+1. We then computed the quantile associated to each ratio under the standard normal distribution using the R function QNORM, yielding Gaussian distributed residuals for lifespan. LRS was modelled in a generalised linear mixed-effects model with negative binomial distribution and a log link function. Because LRS modelled in such a way showed zero-inflation, we also model the LRS using a hurdle model. There, we used: 1) a logistic GLMM to predict if individuals had any offspring during their lifetime (i.e., a binary event), and 2) a zero-truncated negative binomial GLMM to predict the LRS for individuals that did produce offspring.

All models contained the following fixed-effect predictors: F_{GRM}, sex, social rank and clan size. F_{GRM} was included to estimate whether there was evidence for inbreeding depression on each measure of fitness. The regression of inbreeding coefficients on fitness estimates the strength of inbreeding depression in a population [67–70]. Social rank was defined as individuals' mothers ordinal social rank at birth among native individuals of at least one year of age (i.e., subadults, natal and philopatric individuals) and included in the models to account for reproductive skew caused by the linear social hierarchy that hyenas live in [36,44,71]. Although social ranks can fluctuate throughout an individual's life, we chose to rely on birth rank since the rank hyenas inherit from their mother at the time of birth is known to have a "silver-spoon" effect on fitness throughout the hyena's life [45,72]. We rely on ordinal rank since it is the rank metric that best predicts most fitness components in the focal population [73]. Although we only required the social ranks of mothers, natal, subadult and philopatric males were kept in the hierarchies since these males compete with females for resources. Cubs (i.e., individuals between 0 and 12 months of age) were excluded from the hierarchies because dominance relationships among younger hyenas are unstable [74]. Immigrants were also excluded since they generally submit to all native individuals [75]. Clan size was defined as the estimated number of individuals (including cubs and immigrants) that were members of the clan each individual was born into at the time of their birth to account for any effect of density on fitness measures. In addition to these fixed-effect predictors, we included individuals' clan of birth, mother identity and birth year, as random-effect predictors, to account for phenotypic variation between clans (which may be caused by, e.g., density and territory quality), maternal effects, and cohort effects, respectively.

To test if our estimates of inbreeding depression on each measure of fitness were affected by population or relatedness structure [32], we re-fitted the models to include a relatedness matrix as a random effect, thereby accounting for covariance among relatives and estimating additive genetic variance. This matrix was calculated from the population pedigree with the *nadiv* R package [76]. Predicted inbreeding depression did not differ between models that included a relatedness matrix or not (see supplementary material), and we therefore present results from models that were fitted without relatedness information.

To test if the strength of inbreeding depression varies between sexes or according to social rank and clan size, we re-ran the models described above after additionally including the interactions between F_{GRM} and sex, between F_{GRM} and social rank, and between F_{GRM} and clan size. To test if there were differences between clans in the strength of inbreeding depression, we further included a random-slope term that allowed the effect of F_{GRM} to vary among clans.

Finally, to compare whether the estimated effect of inbreeding on fitness, and therefore our inferences about the strength of inbreeding depression in the population, was affected by using a pedigree-based *vs.* a genomic-based inbreeding coefficient, we re-ran each of the main models described (one model per fitness measure) using F_{PED} as a fixed-effect predictor instead of F_{GRM}. We also re-ran these F_{PED} models using the same datasets as with F_{GRM}. This allowed us to compare inbreeding depression assessed by both F_{GRM} and F_{PED} on the exact same data, but this necessarily includes individuals that had much less accurate estimates of F_{PED} because these datasets were not subject to filtering based on individuals having at least three known grandparents (see above).

The statistical significance of selected effects was estimated using likelihood ratio tests by the function ANOVA in *spaMM* [66], with the null distribution of the test statistics generated by parametric bootstrap (1000 iterations). When testing fixed effects, models were fitted using the method ML in *spaMM*. When testing random slopes, we used REML for all fitness components.

To quantify the strength of inbreeding depression, we predicted each fitness component (the probability of juvenile survival, lifespan and LRS) both for $F_{GRM} = 0$ and $F_{GRM} = 0.25$. We did so using a partial-dependence effect approach: we used the fitted LMMs and GLMMs to predict fitness components for all 2393 individuals which presented complete information on all predictor variables (but F_{GRM}), considering, in turn, that they all had $F_{GRM} = 0$ or $F_{GRM} = 0.25$. These predictions, which account for the realisation of the random effects, were then averaged across all 2393 individuals to produce a single fitness value per fitness component-F_{GRM} combination. The approach thus describes the effect of F_{GRM} on each fitness component after accounting for the (average) effects of the other variables [77]. For juvenile survival and LRS those predictions were directly produced using PDEP_EFFECTS in *spaMM*. For lifespan, we had to do this manually since back transforming the predictions before averaging them could not be handled natively due to the custom transformation we applied on the data. Confidence intervals around the predictions were computed accounting for the uncertainty in fixed effects but not random effects (i.e., using the option intervals = "fixefVar" when calling PDEP_EFFECTS) so as to mirror the results from likelihood ratio tests.

Results

Rates of inbreeding were fairly low in the population and close inbreeding events were rare (N_{IND} with $F_{GRM} > 0.1 = 17$ and N_{IND} with $F_{GRM} > 0.05 = 48$, Figure 1A). Genomic (F_{GRM}) and pedigree-based (F_{PED}) inbreeding coefficients were positively correlated (unfiltered F_{PED}: $r = 0.588$, $N = 1119$; filtered F_{PED}: $r = 0.625$, $N = 704$), but F_{GRM} exhibited substantially greater variance than F_{PED} (σ^2 $F_{GRM} = 0.00105$; σ^2 unfiltered $F_{PED} = 0.000399$; σ^2 filtered $F_{PED} = 0.000579$; Fig. 1A), consistent with genomic measures capturing more realised variation in autozygosity. The association between F_{GRM} and social rank at birth or clan size was weak and close to zero (Fig. 1C and 1D). Spatially, average F_{GRM} was similar across clans, with clan means all close to zero (Fig. 1E), indicating little spatial structuring in realised inbreeding. Mean F_{GRM} showed little temporal trend across the study period (Fig. 1F). F_{PED} showed little variation across social rank or clans (Fig. S1 & S2) but increased through time, which reflects increasing pedigree depth over the course of the study.

Inbreeding depression

For juvenile survival, the estimated effect of F_{GRM} was negative (Fig. 2A), and the model predicted that an individual with $F_{GRM} = 0$ had a probability to survive to age two of 0.666 ($CI_{95\%} = 0.575$–0.747), compared to 0.516 ($CI_{95\%} = 0.258$–0.762) for an individual with $F_{GRM} = 0.25$, equating to a survival probability that was 0.143 lower (Fig. 3). However, uncertainty in the effect of inbreeding on juvenile survival was large and the negative trend showed weak statistical support (Table 1, Fig. 2A, $N = 993$). We found evidence for inbreeding depression in both lifespan and LRS as increasing F_{GRM} was associated with shorter lifespan and reduced

LRS (Fig. 2B and 2C, Table 1, N = 768). For lifespan, the model predicted that an individual with $F_{GRM} = 0$ lived on average 5.26 years ($CI_{95\%}$ = 4.17–6.46), compared to 1.74 years ($CI_{95\%}$ = 0.771–4.03) for an individual with $F_{GRM} = 0.25$, equating to 3.52 fewer years in highly inbred individuals (Fig. 3). For LRS, the model predicted that an individual with $F_{GRM} = 0$ produced on average 2.30 offspring ($CI_{95\%}$ = 1.56–3.41), compared to 0.241 offspring ($CI_{95\%}$ = 0.0764–0.759) for an individual with $F_{GRM} = 0.25$, equating to 2.06 fewer (Fig. 3). Alternative modelling (i.e., hurdle modelling, see Method) reveals that inbreeding significantly reduces both the probability to produce any offspring, as well as the average number of offspring for hyenas that did produce offspring (Table S3). These results were robust to the choice of the right-censor date (Fig. S3–5) and to the inclusion of additive genetic variance (V_A) (Fig. S6, Table S4).

Among other fixed-effect predictors, the social rank of the mother at birth exerted a significant negative influence on all fitness components (Table 1), implying that offspring benefited from being born from high-ranking mothers (Table 1). Both clan size and being male also showed a significant negative association with both lifespan and LRS: individuals born into larger clans, and males in general, had shorter lifespans, and produced fewer offspring. No clear association was found between these predictors and juvenile survival (Table 1).

Inbreeding depression across social environments

Sex differences in the strength of inbreeding depression measured using F_{GRM} were inconsistent across fitness components and imprecisely estimated. In all cases, there was no statistical support for sex differences in inbreeding depression (i.e., interactions F_{GRM}:Sex_M in Table 2 were not significant, Fig. S7). We did not find statistical evidence for any interactions between inbreeding depression (measured with F_{GRM}) and social rank or clan size. All point estimates suggest weaker inbreeding depression at the bottom of hierarchies and in large clans, but all confidence intervals broadly overlap zero and with weak statistical support (Table 2, Fig. S8 & S9). Random-slope terms indicated that the between-clan variation in the strength of inbreeding depression was small and non-significant (Fig. S10).

Estimated inbreeding depression using F_{PED}

Across all fitness components, F_{PED} predicted similar estimates of inbreeding depression to F_{GRM}. When using a dataset filtered to only include individuals that had at least three known grandparents (see methods), the effect of F_{PED} on fitness components was consistently negative and of similar magnitude to that predicted by F_{GRM} (Table 3 & S5, Fig. 3 & S11). For juvenile survival, the model predicted that an individual with $F_{PED} = 0$ had a probability to survive to age two of 0.642 ($CI_{95\%}$ = 0.535–0.738), compared to 0.544 ($CI_{95\%}$ = 0.252–0.808) for an individual with $F_{PED} = 0.25$, equating to a survival probability that was 0.0982 lower in inbred individuals (Fig. 3). For lifespan, the model predicted that an individual with $F_{PED} = 0$ lived on average 4.73 years ($CI_{95\%}$ = 3.65–5.98), compared to 0.73 years ($CI_{95\%}$ = 0.273–2.15) for an individual with $F_{PED} = 0.25$, equating to 4.00 fewer years (Fig. 3). For LRS, the model predicted that an individual with $F_{PED} = 0$ produced on average 2.17 offspring ($CI_{95\%}$ = 1.49–3.18), compared to 0.116 offspring ($CI_{95\%}$ = 0.0173–0.781) for an individual with $F_{PED} = 0.25$, equating to 2.05 fewer (Fig. 3). Interestingly, however, when we ran F_{PED} models with the same dataset used for the F_{GRM} models presented above, we found that estimates differed widely except for LRS (albeit with larger associated error) (Table S5). Comparative analyses that included SNP-genotyped individuals that also had at least three known grandparents yielded similar results but with wider confidence intervals owing to the reduced sample size.

Discussion

Our study provides evidence for inbreeding depression in lifetime fitness components in a population of spotted hyenas, adding to a growing body of literature documenting the widespread occurrence of inbreeding depression in social mammals [7,26,68,78]. While close inbreeding events were rare, even modest increases in inbreeding had measurable negative effects on fitness. We found that individuals with higher inbreeding coefficients exhibited shorter lifespans and produced fewer offspring, consistent with the expectation that increased homozygosity exposes deleterious recessive alleles, prevents heterozygote advantage, and thus reduces overall fitness [3,69]. Importantly, we found only subtle and imprecise evidence that inbreeding depression was moderated by the social environment. Finally, pedigree-based estimates were comparable to genomic inbreeding coefficients in their power to detect inbreeding depression, but only when when individuals with incomplete ancestry were excluded.

All fitness components we investigated were negatively impacted by inbreeding. However, inbreeding depression was weak and non-significant for juvenile survival. This contrasts with many studies of inbreeding depression in mammals, where early-life survival is among the components of fitness that are the most sensitive to inbreeding depression [26,78,79]. One explanation could be that our data underrepresent inbreeding-related mortality occurring very early in life and thus before first observation [80] because hyenas were typically first observed at a mean age of 3.11 months (SD = 3.50 months, median = 1.97 months). Alternatively, environmental factors early in life, such as extensive parental care or communal denning, may buffer the costs of being inbred [21]. Nonetheless, the detected costs of inbreeding on later-life fitness components (i.e., in lifespan and LRS) suggest that inbreeding depression in this population may be more strongly expressed in adulthood.

Despite well-established links between social dominance, clan size and fitness in spotted hyenas [38,44,45,81], we found little evidence that the strength of inbreeding depression varied systematically across social ranks or as a function of clan size. Although social rank strongly influences fitness in the monitored population, rates of inbreeding did not vary significantly across the hierarchy, and the strength of inbreeding depression was broadly consistent. The same was true of clan size. Additionally, while mean fitness differed among social groups (i.e., clans), we found no evidence that the strength of inbreeding depression varied between clans. This is notable given that clans differ in ecological variables which could theoretically alter the fitness costs of inbreeding (e.g., in habitat, food availability, demographic history, adaptive potential and sex ratio [40,46,47]). Together, these results therefore suggest that core features of the social environment, which are central determinants to fitness in hyenas, do not strongly modulate inbreeding depression in this species.

Comparing genomic (F_{GRM}) and pedigree-based (F_{PED}) estimates of inbreeding depression, we found that pedigree-based estimates predicted comparable inbreeding depression in all fitness components to F_{GRM}, but only when considering individuals of well-known ancestry. Pedigree-based estimates only predicted significant inbreeding depression for LRS when applied to SNP-genotyped individuals. This highlights one of the core limitations of using pedigree based inbreeding estimates: they require known ancestry across multiple generations to accurately estimate inbreeding. In contrast, F_{GRM} reflects actual genome-wide homozygosity and offers greater resolution, especially in natural populations with incomplete or shallow pedigrees. Typically, the goal of genomic based inbreeding coefficients is to measure the realised autozygosity caused by inbreeding, for which runs of homozygosity (ROH) are considered optimal [29,30]. However, ROH cannot be captured without known SNP positions, which are not available for hyenas or many species of conservation concern. In these cases, researchers need to use alternative measures, and in fact F_{GRM} has been found to be more informative when a population is genetically structured [31,32]. Our study adds to this evidence, suggesting that F_{GRM} may be reliable in its estimation of inbreeding depression in wild species without a highly contiguous genome assembly. Our results also suggest that in systems where there are multi-generation pedigrees, filtering for known ancestry may be an appropriate step to estimating inbreeding depression reliably in the absence of genomic data.

Taken together, our results highlight the value of long-term, individual-based studies that integrate molecular and demographic data to investigate inbreeding depression. By combining genomic tools with detailed life-history records, we uncover subtle patterns of inbreeding and its cumulative effect on fitness. Interestingly, the social environments had little impact on the strength of inbreeding depression, despite its well-documented effects on fitness in this species. It is important to note, however, that even if some main components of the social environment that could moderate inbreeding depression across a hyena's lifetime were included (social rank, clan size), others are not addressed in this study (e.g., social network) and these factors may yet interact with inbreeding to shape fitness outcomes. More broadly, our study adds to growing evidence that inbreeding depression is not restricted to small or isolated populations but can also occur in large, growing populations. While male-biased dispersal, female mate choice, and social structure may reduce the incidence of close inbreeding in spotted hyenas, even low to moderate levels were associated with large fitness costs. These findings have general implications for conservation and management: maintaining gene flow and minimizing inbreeding remain important, even in populations that appear demographically healthy [82]. Future work should examine how ecological variability, kin structure, and mate-choice behaviours interact to influence inbreeding risk and its fitness consequences over time.

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Ethics

All study procedures were performed in compliance with ethical regulations outlined by the Internal Committee for Ethics and Animal Welfare of the Leibniz Institute for Zoo and Wildlife Research Berlin (Permit No. 2020-06-02).

Competing interests

The authors have no competing interests to declare

Data, code and materials

The raw sequencing data are deposited in the Genbank with the NCBI BioProject accession no. PRJNA951614. Code used for bioinformatic analyses of genetic data can be found at: and

https://git.imp.fu-berlin.de/begendiv/radseq-preprocessing-pipeline/-/blob/main/scripts/digestion/checkRestrictionSites.py). R code and data needed to reproduce analyses and results, including all tables and figures, can be found at https://github.com/hyenaproject/inbreeding_depression_2026.

Use of Artificial Intelligence (AI) and AI-assisted technologies

No AI technologies were used in the preparation of this article.

Author contributions

Conceptualisation: KS, LEBK, AC; Data curation: OPH, ED, LSA; Methodology: KTC, LFW, LSA, AC, JMP, KS; Resources: OPH, ED, LSA, AC; Software: KTC, AC, KS, LFW, LSA; Investigation: PN, OPH, ED, LSA; Formal analysis: KTC, KS, AC, LSA; Visualisation: KTC, KS, AC; Validation: KTC, LFW, AC, JMP, KS; Writing – original draft: KTC, KS; Writing – review and editing: all; Supervision: KS, JMP, AC; Project administration: KS, LEBK; Funding acquisition: LEBK, OPH.

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Tables and Figures

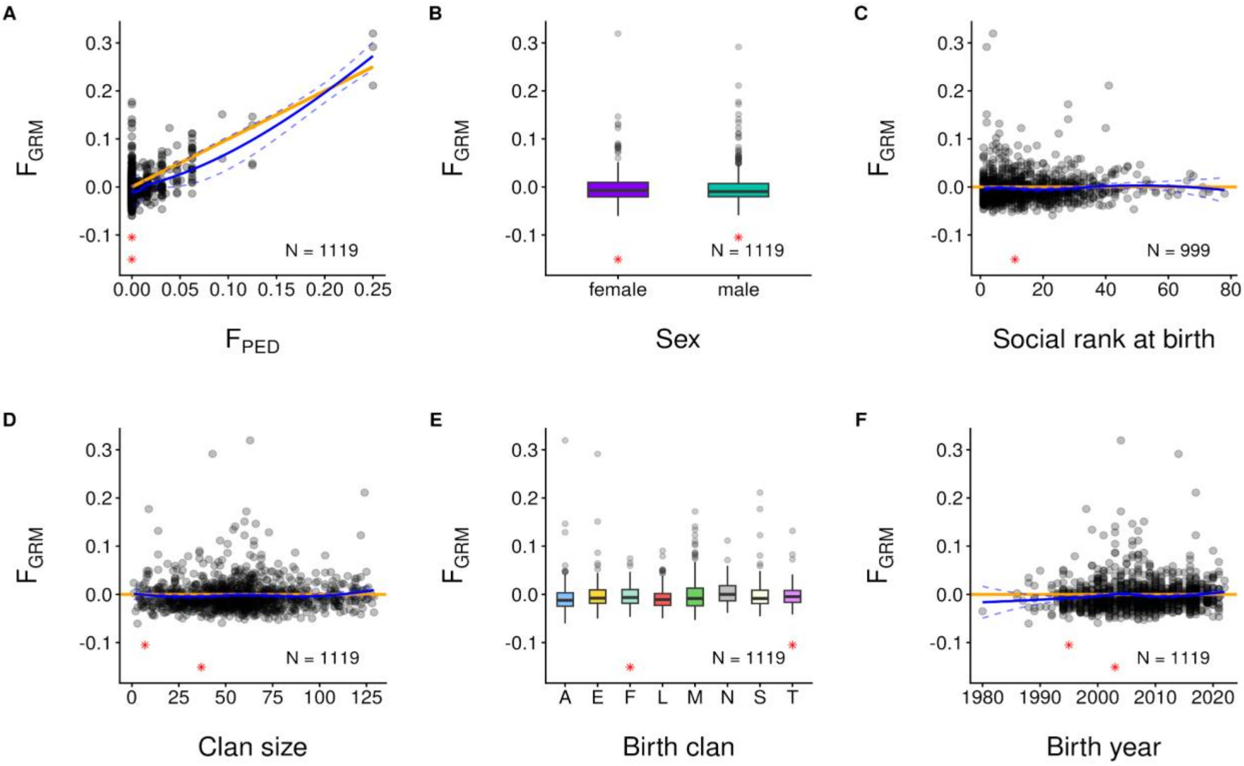

Figure 1. Inbreeding in spotted hyenas of Ngorongoro crater, Tanzania. (A) Relationship between a pedigree based inbreeding coefficient (F_{PED}) and genomic inbreeding coefficient (F_{GRM}) measured as $\hat{F}^{III}$. (B–F) Relationship between individuals' F_{GRM} and: their sex (B); their maternal ordinal social rank at birth (ranging from 1 to *n* where *n* is the number of native individuals in the clan, and where 1 indicates highest social rank) (C); the number of clan members at their birth (D), the clan where they were born (E), and their year of birth (F). The plotted orange line shows the line of equation y = x in A and y = 0 in C, D, F. In A, C, D, F, the blue full line depicts the prediction from a local polynomial regression (i.e., loess) with its associated standard error shown as dotted lines. In B and E, boxes represent interquartiles, thick horizontal lines represent medians, whiskers extend to extreme observations no more than 1.5× the interquartile range, and grey points represent more extreme observations still. The red asterisks depict two individuals with extremely low F_{GRM} values that were discarded from the analyses. The sample size is indicated and refers to the number of distinct individuals.

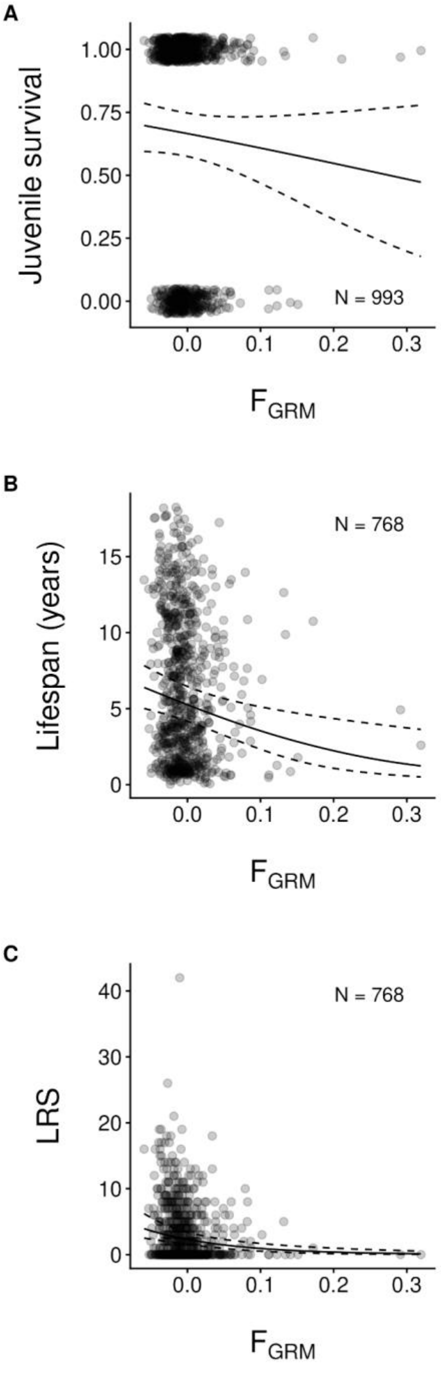

Figure 2. Predicted relationship between inbreeding, measured as F_{GRM}, and three measures of fitness: (A) juvenile survival, measured as survival to 24 months, (B) lifespan in years, and (C) lifetime reproductive success (LRS). Data points show raw data (vertically jittered in the case of juvenile survival). Solid lines show the predicted effect of F_{GRM} on each trait, predicted as partial-dependence effects across the empirical distribution of all other fixed effects and marginal to the random effects and back-transformed to the original data scale. Dotted lines show 95% confidence intervals.

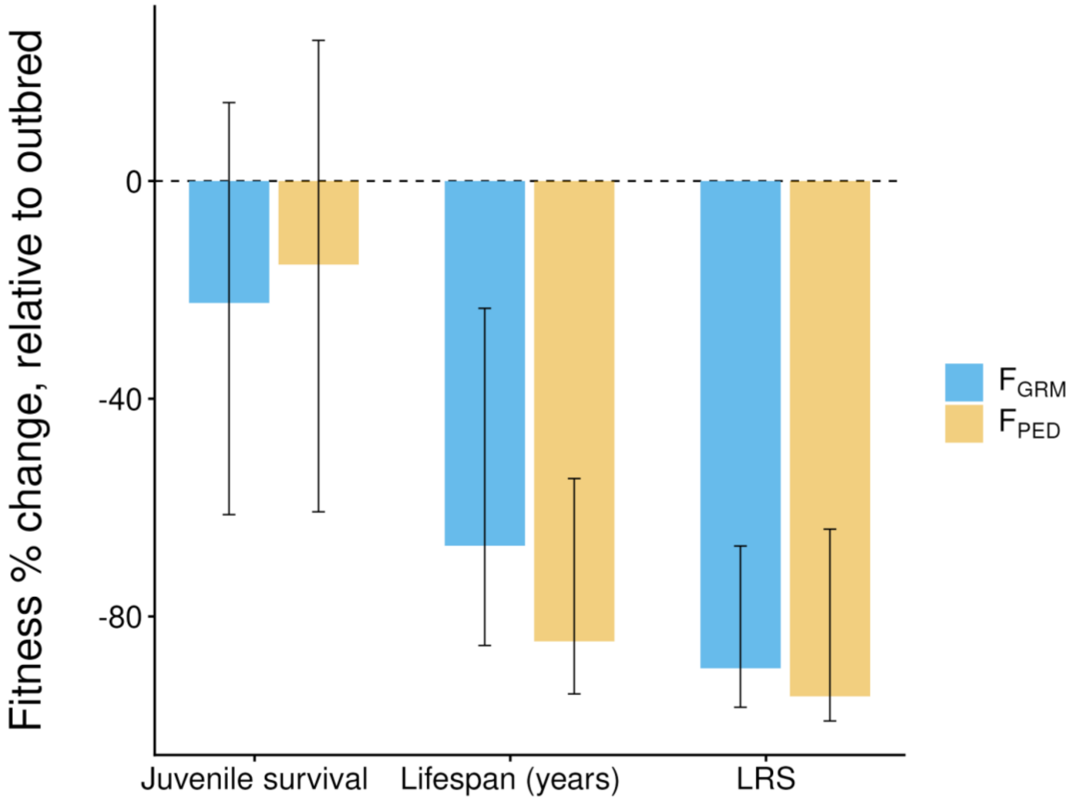

Figure 3. A comparison of the predicted inbreeding depression from F_{GRM} (blue) and F_{PED} (yellow) for three measures of fitness. Inbreeding depression is estimated as the predicted percentage change in trait values when comparing a fully outbred individual ($F = 0$) as reference ($y = 0$) to individuals with $F = 0.25$. Predictions correspond to partial-dependence effects across the empirical distribution of all other fixed effects and marginal to the random effects and back-transformed to the original data scale. Bars show the point predictions with error bars representing 95% confidence intervals.

Table 1. Parameter estimates and conditional standard errors (SE) for fixed effects and random effects variances fitted in linear mixed-effects models used to estimate the effect of F_{GRM} on three measures of fitness: lifetime reproductive success (LRS), survival to two years (juvenile survival) and lifespan. All parameter estimates are shown on a link scale, with a rank-based transformation applied to lifespan (see Methods). P-values (*P*) for fixed effects were calculated using LRT tests producing a chi-square statistic (Chi2).

Trait		**Parameter**	**Estimate**	**SE**	**Chi2**	***P***
Juvenile survival	Fixed Effects (*β*)	Intercept	1.788	0.306	-	-
		F_{GRM}	-2.987	2.481	1.323	0.261
		Sex_M	-0.009	0.154	0.003	0.964
		Social rank	-0.047	0.007	43.149	0.001
		Clan size	-0.002	0.004	0.178	0.686
	Random Effects (*V*)	Birth clan	0.037	-	-	-
		Mother	0.203	-	-	-
		Birth year	0.567	-	-	-
Lifespan	Fixed Effects (*β*)	Intercept	1.047	0.110	-	-
		F_{GRM}	-2.658	0.914	8.325	0.006
		Sex_M	-0.126	0.058	4.600	0.028
		Social rank	-0.011	0.003	12.068	0.002
		Clan size	-0.008	0.002	10.349	0.003
	Random Effects (*V*)	Birth clan	0.019	-	-	-
		Mother	0.052	-	-	-
		Birth year	0.002	-	-	-
LRS	Fixed Effects (*β*)	Intercept	2.290	0.260	-	-
		F_{GRM}	-9.036	2.173	15.777	0.001
		Sex_M	-0.368	0.127	7.691	0.007
		Social rank	-0.030	0.006	23.734	0.001
		Clan size	-0.017	0.004	8.107	0.004
	Random Effects (*V*)	Birth clan	0.141	-	-	-
		Mother	< 0.001	-	-	-
		Birth year	0.051	-	-	-

Table 2. Parameter estimates and conditional standard errors (SE) for fixed effects and random effects variances fitted in linear mixed-effects models used to estimate how the effect of F_{GRM} on three measures of fitness varies between sexes, or across social ranks, clan size and between clans. All parameter estimates are shown on a link scale, with a rank-based transformation applied to lifespan (see Methods). A colon (":") represents an interactive effect and a vertical line (|) represents a random slope term. P-values (*P*) for interaction terms were calculated using LRT tests producing a chi-square statistic (Chi2).

Trait		**Parameter**	**Estimate**	**SE**	**Chi2**	***P***
Juvenile survival	Fixed Effects (*β*)	Intercept	1.817	0.310	-	-
		F_{GRM}	-11.854	7.024	-	-
		Sex_M	-0.007	0.155	-	-
		Social rank	-0.048	0.007	-	-
		Clan size	-0.002	0.004	-	-
		F_{GRM}:Sex_M	-6.707	4.952	1.728	0.204
		F_{GRM}:Social rank	0.158	0.225	0.455	0.498
		F_{GRM}:Clan size	0.172	0.114	2.127	0.159
	Random Effects (*V*)	Clan	0.041	-	-	-
		Clan|F_{GRM}	0.043	-	0.047	0.749
		Mother	0.199	-	-	-
		Birth year	0.592	-	-	-
Lifespan	Fixed Effects (*β*)	Intercept	1.038	0.111	-	-
		F_{GRM}	-6.165	3.180	-	-
		Sex_M	-0.118	0.059	-	-
		Social rank	-0.011	0.003	-	-
		Clan size	-0.008	0.002	-	-
		F_{GRM}:Sex_M	0.944	1.841	0.241	0.615
		F_{GRM}:Social rank	0.032	0.092	0.119	0.740
		F_{GRM}:Clan size	0.047	0.054	0.643	0.437
	Random Effects (*V*)	Clan	0.020	-	-	-
		Clan|F_{GRM}	0.061	-	0.071	0.795
		Mother	0.053	-	-	-
		Birth year	0.001	-	-	-
LRS	Fixed Effects (*β*)	Intercept	2.276	0.258	-	-
		F_{GRM}	-7.455	6.369	-	-
		Sex_M	-0.398	0.128	-	-
		Social rank	-0.030	0.006	-	-
		Clan size	-0.017	0.004	-	-
		F_{GRM}:Sex_M	-6.317	4.440	2.019	0.142
		F_{GRM}:Social rank	0.122	0.221	0.295	0.566
		F_{GRM}:Clan size	0.014	0.111	0.011	0.916
	Random Effects (*V*)	Clan	0.132	-	-	-
		Clan|F_{GRM}	1.755	-	0.158	0.716
		Mother	< 0.001	-	-	-
		Birth year	0.052	-	-	-

Table 3. Parameter estimates and conditional standard errors (SE) for fixed effects fitted and random effects variances in linear mixed-effects models used to estimate the effect of F_{PED} on three measures of fitness. All parameter estimates are shown on a link scale, with a rank-based transformation applied to lifespan (see Methods). P-values (*P*) for fixed effects were calculated using LRT tests producing a chi-square statistic (Chi2).

Trait		**Parameter**	**Estimate**	**SE**	**Chi2**	***P***
Juvenile survival	Fixed Effects (*β*)	Intercept	1.993	0.332	-	-
		F_{PED}	-1.929	2.919	0.418	0.537
		Sex_M	-0.026	0.130	0.037	0.854
		Social rank	-0.037	0.006	43.313	0.001
		Clan size	-0.011	0.004	7.845	0.007
	Random Effects (*V*)	Birth clan	0.256	-	-	-
		Mother	0.156	-	-	-
		Birth year	0.356	-	-	-
Lifespan	Fixed Effects (*β*)	Intercept	0.671	0.118	-	-
		F_{PED}	-4.417	1.307	11.261	0.004
		Sex_M	-0.099	0.054	3.338	0.069
		Social rank	-0.012	0.002	22.755	0.001
		Clan size	-0.003	0.002	2.227	0.165
	Random Effects (*V*)	Birth clan	0.013	-	-	-
		Mother	0.019	-	-	-
		Birth year	0.027	-	-	-
LRS	Fixed Effects (*β*)	Intercept	1.807	0.280	-	-
		F_{PED}	-11.707	3.927	8.370	0.007
		Sex_M	-0.455	0.143	9.801	0.003
		Social rank	-0.043	0.006	39.573	0.001
		Clan size	-0.005	0.004	1.207	0.292
	Random Effects (*V*)	Birth clan	0.036	-	-	-
		Mother	< 0.001	-	-	-
		Birth year	0.157	-	-	-