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A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal

David A. Wells[1,2] | Michael A. Cant[3] | Hazel J. Nichols[2†] | Joseph I. Hoffman[1†]

[1]Department of Animal Behaviour, University of Bielefeld, Bielefeld, Germany

[2]School of Natural Science and Psychology, Liverpool John Moores University, Liverpool, UK

[3]College of Life and Environmental Sciences, University of Exeter, Penryn, UK

Correspondence
David A. Wells, Department of Animal Behaviour, University of Bielefeld, Bielefeld, Germany.
Emails: david.wells@uni-bielefeld.de; d.a.wells@2016.ljmu.ac.uk

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Abstract

Inbreeding depression, the reduced fitness of offspring of closely related parents, is commonplace in both captive and wild populations and has important consequences for conservation and mating system evolution. However, because of the difficulty of collecting pedigree and life-history data from wild populations, relatively few studies have been able to compare inbreeding depression for traits at different points in the life cycle. Moreover, pedigrees give the expected proportion of the genome that is identical by descent (IBD_g) whereas in theory with enough molecular markers realized IBD_g can be quantified directly. We therefore investigated inbreeding depression for multiple life-history traits in a wild population of banded mongooses using pedigree-based inbreeding coefficients (f_{ped}) and standardized multilocus heterozygosity (sMLH) measured at 35–43 microsatellites. Within an information theoretic framework, we evaluated support for either f_{ped} or sMLH as inbreeding terms and used sequential regression to determine whether the residuals of sMLH on f_{ped} explain fitness variation above and beyond f_{ped}. We found no evidence of inbreeding depression for survival, either before or after nutritional independence. By contrast, inbreeding was negatively associated with two quality-related traits, yearling body mass and annual male reproductive success. Yearling body mass was associated with f_{ped} but not sMLH, while male annual reproductive success was best explained by both f_{ped} and residual sMLH. Thus, our study not only uncovers variation in the extent to which different traits show inbreeding depression, but also reveals trait-specific differences in the ability of pedigrees and molecular markers to explain fitness variation and suggests that for certain traits, genetic markers may capture variation in realized IBD_g above and beyond the pedigree expectation.

KEYWORDS
banded mongoose, cooperative breeding, heterozygosity, inbreeding depression, microsatellites, reproductive success, survival

1 | INTRODUCTION

Inbreeding depression, the reduction in offspring fitness that can result from incestuous matings, occurs in a wide range of both captive and wild populations (Hedrick & Garcia-Dorado, 2016; Keller &

†Joint senior authors.

Waller, 2002). Inbreeding increases the proportion of the genome that is identical by descent (IBD_g), which in turn reduces fitness mainly through the increased expression of deleterious recessive alleles but also due to increased homozygosity at loci showing overdominance (Charlesworth & Willis, 2009). The resulting loss of fitness can be substantial and is believed to have shaped the evolution of dispersal and mating behaviour in many species. Consequently, quantifying the severity of inbreeding depression in natural populations is essential for understanding population and evolutionary dynamics (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002; Nichols, 2017; Szulkin, Stopher, Pemberton, & Reid, 2013).

Inbreeding depression is predicted to be strongest for traits that are closely related to fitness such as survival and reproduction, as these will be subject to stronger directional selection and therefore exhibit greater directional dominance (Falconer & Mackay, 1996). This is supported by a meta-analysis of 54 animal species, although most of the studies involved were of captive or experimental populations (DeRose & Roff, 1999). However, understanding how inbreeding depression affects different life-history traits in natural populations is more challenging due to the difficulty of collecting high-quality lifetime fitness measures and generating deep, well-resolved pedigrees. Furthermore, strong viability selection against inbred offspring will result in an adult population in which inbred individuals are rare, potentially making it more difficult to detect inbreeding depression for late-acting traits (Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016).

Traditionally, pedigrees were considered the gold standard for measuring inbreeding in natural populations (Pemberton, 2004). However, the vast majority of pedigrees are incomplete and will also contain errors that can impair their ability to detect inbreeding depression (Reid et al., 2014; Taylor, Kardos, Ramstad, & Allendorf, 2015). Additionally, pedigrees cannot account for inbreeding caused by ancestors who are not included in the pedigree. This can result in downwardly biased estimates of inbreeding, particularly where the pedigree is only a few generations deep and relationships among the founders are unknown (Kardos, Luikart, & Allendorf, 2015). Arguably, an even greater issue is that pedigrees simply cannot be generated for the majority of wild populations, many of which are large and demographically open.

A further drawback of pedigrees is that, even when multiple generations of accurate ancestry data can be collected, the pedigree inbreeding coefficient (f_{ped}) quantifies an individual's *expected* IBD_g based on the known common ancestors of its parents, whereas *realized* IBD_g will differ stochastically from this expectation due to Mendelian segregation and recombination (Hedrick, Kardos, Peterson, & Vucetich, 2016; Hill & Weir, 2011; Knief, Kempenaers, & Forstmeier, 2017). The variance in realized IBD_g among individuals with the same f_{ped} will be higher for species with few chromosomes and short genetic maps (Fisher, 1965; Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015) and will also decrease with the number of generations separating an inbred individual from its common parental ancestor(s) as IBD chromosomal segments are gradually broken down by successive recombination events (Hedrick et al., 2016).

As deep, high-quality pedigrees are also lacking for the majority of natural populations, many studies have used the heterozygosity of small panels of typically around 10–20 presumed neutral markers such as microsatellites as a surrogate measure of IBD_g. The result is a large and expanding literature describing heterozygosity-fitness correlations (HFCs) covering a long list of traits and species (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009). However, estimates of IBD_g based on such small panels of markers will tend to have limited precision due to both high sampling variance and the difficulty of distinguishing identity by descent (IBD) from identity by state (IBS, Balloux, Amos, & Coulson, 2004; Slate et al., 2004). Recent simulation and empirical studies suggest that these issues can be overcome with very large panels of markers, with around ten thousand or more single nucleotide polymorphisms (SNPs) being preferable under most circumstances even to a deep pedigree for quantifying inbreeding depression (Hoffman et al., 2014; Huisman et al., 2016; Kardos et al., 2015; Wang, 2016). However, until SNP genotyping costs fall to the point where such large data sets can be collected within the budgets of most projects, it is likely that microsatellites will continue to be used to investigate inbreeding effects in wild populations.

Only a handful of studies have directly compared the ability of f_{ped} and microsatellites to detect inbreeding depression (e.g., Grueber, Waters, & Jamieson, 2011; Taylor et al., 2010), and these have uncovered mixed results. At one end of the spectrum, Nietlisbach et al. (2017) used an unusually deep and well-resolved song sparrow pedigree to show that f_{ped} outperformed microsatellite heterozygosity, even when the latter could be calculated from an unusually large panel of 160 markers. At the other end, both Forstmeier, Schielzeth, Mueller, Ellegren, and Kempenaers (2012) and Hammerly, Morrow, and Johnson (2013) found that smaller panels of around ten microsatellites explained more fitness variation than f_{ped}. These contradictory outcomes probably reflect a multitude of factors including variation among studies in pedigree depth and quality, marker number and resolution, as well as factors intrinsic to a given system such as the recombination landscape. Consequently, in order to obtain a more general picture of how pedigrees and genetic markers can capture fitness variation, similar studies of a wider variety of taxa are needed.

A related question is whether the heterozygosity of genetic markers can explain fitness variation above and beyond that explained by f_{ped}. Some studies have approached this question by testing for HFCs within individuals of the same pedigree inbreeding class (Hansson, Westerdahl, Hasselquist, Åkesson, & Bensch, 2004; Hemmings, Slate, & Birkhead, 2012), while others have constructed statistical models of the focal traits containing both f_{ped} and marker heterozygosity (e.g., Bensch et al., 2006), an approach that Nietlisbach et al. (2017) recently termed "residual heterozygosity-fitness correlation." However, if these two inbreeding measures are strongly correlated, the variance explained by either term cannot be properly

partitioned due to collinearity (Dormann et al., 2013). One way to account for this would be to take the residuals of marker heterozygosity on f_{ped} and fit this as an explanatory variable alongside f_{ped}. The variance shared by these two terms will be attributed to the pedigree, while any effect of residual heterozygosity will reflect the ability of the markers to detect variation in realized IBD_g that cannot be captured by the pedigree. This approach is known as "sequential regression" (Graham, 2003) or sometimes "residual regression" and has been shown to perform well in a comparison of approaches for dealing with collinearity (Dormann et al., 2013).

A long-term study of banded mongooses (*Mungos mungo*) provides an excellent opportunity to investigate the strength of inbreeding depression for multiple traits, as well as to explore the ability of f_{ped} and marker heterozygosity to capture fitness variation in a wild vertebrate population. Banded mongooses live in social groups of 10–40 adults and, unlike most cooperative breeders, members of both sexes habitually breed within their natal pack despite the presence of close relatives (Nichols, Cant, Hoffman, & Sanderson, 2014). As a result, inbreeding appears to be common despite evidence that females attempt to avoid inbreeding and that males preferentially mate guard more distant relatives (Sanderson, Wang, Vitikainen, Cant, & Nichols, 2015). Furthermore, inbreeding appears to have fitness implications for offspring as recent studies have uncovered inbreeding depression for both yearling body mass and parasite load (Mitchell, Vitikainen, Wells, Cant, & Nichols, 2017; Sanderson et al., 2015). However, although both of these studies were based on a high-quality, nine-generation deep pedigree, only the latter compared the ability of f_{ped} and microsatellite heterozygosity to detect inbreeding depression.

Here, we genotyped an additional 192 individuals at 35 microsatellite loci in order to enlarge the existing banded mongoose pedigree to include 777 individuals with all four grandparents known. The resulting data set was then used to investigate inbreeding depression for a variety of traits acting at different time points in the life cycle: (i) survival to nutritional independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv) annual reproductive success. We additionally evaluated the abilities of f_{ped}, marker heterozygosity and residual marker heterozygosity to detect inbreeding depression. We hypothesized that viability selection against inbred individuals would reduce both the mean and variance in inbreeding in the adult population, thereby rendering inbreeding depression for late-acting traits more difficult to detect. We also hypothesized that, despite having a high-quality pedigree, our moderately large panel of microsatellites would allow us to explain fitness variation above and beyond that explained by f_{ped} and that the explanatory power of the markers would increase with the number of loci.

2 | MATERIALS AND METHODS

2.1 | Study site, individual identification and sample collection

This study was conducted on a free-ranging population of banded mongooses in Queen Elizabeth National Park, Uganda (0°12′S, 27°54′E). The study area comprises approximately 10 km^2 of savannah on and around the Mweya Peninsula and a weather station near the centre measures the amount of daily rainfall. Genetic, behavioural and life-history data were collected from a total of 1,978 individuals between May 1997 and July 2016 inclusive. At any one time, the population consisted of approximately 250 individuals belonging to 10–12 social groups. A combination of approaches was used to identify individuals in the field. The majority of individuals were first captured as pups and given either a unique tattoo or a subcutaneous pit tag (TAG-P-122IJ, Wyre Micro Design Ltd., UK) to allow permanent identification. For genetic analysis, a 2-mm tissue sample was taken from the tip of the tail using surgical scissors and a dilute solution of potassium permanganate was applied to minimize infection risk. To identify individual mongooses by sight, commercially available hair dye (L'Oreal, UK) was used to apply unique patterns to animals up to 6 months of age. Adults were given a unique shave pattern and, after they had stopped growing, were fitted with colour-coded plastic collars. To maintain dye markings, shave patterns and collars, all individuals were trapped every 3–6 months as described by Cant (2000), Hodge (2007) and Jordan, Mwanguhya, Kyabulima, Rüedi, and Cant (2010).

2.2 | Life-history data collection

Detailed behavioural and life-history data were collected by visiting each pack every 2–4 days. All individuals in the population were habituated to human observers. Mongoose packs could be reliably located because one or two adults in each pack were fitted with a 27-g radio collar (<2% of body mass, Sirtrack Ltd., New Zealand) with a 20-cm whip antenna (Biotrack Ltd., UK). Age could be determined for the majority of individuals born within the study site based on their mother's parturition dates, but was unknown for immigrants. Individual lifespan was calculated as the time in days between the date of birth and the date of death. Death could be distinguished from dispersal because mongooses disperse in groups (Cant, Otali, & Mwanguhya, 2001) and dispersal events are also generally preceded by a period of aggression from the rest of the group (Thompson et al., 2016).

Escorting is a form of care unique to banded mongooses that affects offspring fitness (Cant, Vitikainen, & Nichols, 2013; Gilchrist, 2004; Hodge, 2005). Escorting begins approximately 27 days after birth, when pups leave the den and begin to forage with the pack (Gilchrist, 2004). During this time, some of the pups form an exclusive one-to-one relationship with an adult who feeds, grooms, carries and protects them from predators. We therefore collected detailed data on escorting behaviour so that we could incorporate escorting into our analyses of early-acting fitness traits. Throughout the escorting period, which lasts approximately 2 months, we visited packs once or twice daily. If an adult was closely associated with a pup (i.e., spent more than half of a 20-min observation period within 0.5 m of the focal pup), the adult was deemed to be an escort for that pup. For each pup, we quantified the amount of care received as the proportion of visits during which a pup was seen with an escort.

2.3 | Ethical statement

Research was carried out under licence from the Uganda National Council for Science and Technology, and all procedures were approved by the Uganda Wildlife Authority. All research procedures adhered to the ASAB Guidelines for the Treatment of Animals in Behavioural Research and Teaching and were approved by the Ethical Review Committee of the University of Exeter. Our trapping procedure has been used over 8,000 times, and tissue samples have been taken from over 1,900 individuals with no adverse effects.

2.4 | DNA extraction and microsatellite genotyping

Prior to this study, genetic data were available for 1,748 individuals that were tissue sampled between 1997 and 2013 and genotyped at up to 43 microsatellite loci (Sanderson et al., 2015). All of these loci are known to be in Hardy–Weinberg and linkage equilibrium in the study population (Sanderson et al., 2015). To enlarge this data set, we genotyped an additional 192 individuals that were sampled between 2014 and 2015 at 35 of these microsatellites. We excluded eight loci that had previously been amplified individually and visualized through radioactive incorporation but which failed to amplify reliably in multiplexed PCRs using fluorescent labelled primers. DNA was extracted using Qiagen® DNeasy blood and tissue kits following the manufacturer's protocol. The genotyping was conducted as described in detail by Sanderson et al. (2015). Briefly, fluorescently labelled microsatellite primers were incorporated into seven separate multiplexes. PCR reactions were conducted using a Type It kit (Qiagen) according to the manufacturer's protocol with an annealing temperature of 57°C and a reaction volume of 12 μl. PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer, and allele sizes were scored using GENEMARKER version 1.95 (SoftGenetics, Pennsylvania, USA).

2.5 | Pedigree construction

The resulting microsatellite data set was used to update an existing banded mongoose pedigree, comprising 1,748 individuals genotyped at 35–43 microsatellite loci (Sanderson et al., 2015). We followed the protocol of Sanderson et al. (2015) to extend the pedigree using a combination of MASTERBAYES (Hadfield, Richardson, & Burke, 2006) and COLONY (Jones & Wang, 2010). MASTERBAYES was used as the primary parentage assignment program because of its ability to incorporate phenotypic data, which can result in larger numbers of higher confidence assignments. COLONY was used both to confirm the MASTERBAYES assignments and to assign sibships among individuals with one or both unsampled parents. The latter provides putative information about the relationships among founders and immigrants rather than assuming that they are unrelated.

For the MASTERBAYES analysis, we specified the following strict requirements for assigning parentage: (i) fathers had to be alive on the estimated date of conception of the focal pup; (ii) mothers had to be alive on the date of birth and present in the pack where the focal pup was born; (iii) both parents had to be at least 6 months of age during the month of conception of the focal pup; (iv) offspring could not be their own parents. To maximize confidence in parentage assignments, we also incorporated the following phenotypic data: (i) age and age^2, as reproduction increases with age before tailing off later in life (Sanderson et al., 2015); (ii) whether a female was recorded as having given birth within 4 weeks of the month in which the pup was born; (iii) whether the male was present in the offspring's pack during the month of conception. MASTERBAYES was run for 9,772,000 iterations with a burn in of 750,000 and a thinning interval of 9,022. In order to keep the Metropolis–Hastings acceptance rate between 0.2 and 0.5, the tuning parameters were set to tunePed (beta = 0.3, USdam = 0.03, USsire = 0.03). Successive samples from the posterior distribution had low autocorrelation ($r < .1$). MASTERBAYES parentage assignments were accepted if they had an associated probability greater than or equal to 0.8, although the average assignment probability was 0.99.

Additionally, COLONY was used to assign individuals to full- and half-sibship groups. Candidate parent and exclusion parent lists for input into COLONY were generated using the same criteria as for MASTERBAYES. No maternal or paternal sibships were excluded. We specified a sibship prior of 1.5 for both maternal and paternal average sibship size. This was based on prior knowledge of the breeding system and helped to prevent COLONY from incorrectly grouping offspring into large clusters of false siblings. The probability of a true parent being in the candidate list was set to 0.8, and COLONY assignments were only accepted if they had a probability greater than or equal to 0.8. MASTERBAYES parentage assignments were accepted first, and COLONY assignments were then added where MASTERBAYES failed to confidently assign parentage.

2.6 | Derivation of pedigree *f* and multilocus heterozygosity

Based on the final pedigree, which incorporated information on putative relationships among founders as described above, pedigree inbreeding coefficients (f_{ped}) were calculated for all individuals using the R package PEDANTICS (Morrissey, 2014). However, subsequent analyses involving f_{ped} were based only on individuals with all four grandparents assigned. From the microsatellite data, we also quantified each individual's standardized multilocus heterozygosity (sMLH) using INBREEDR (Stoffel et al., 2016). The same program was also used to calculate g_2, a quantity that estimates identity disequilibrium (the extent to which heterozygosities are correlated across loci) following David, Pujol, Viard, Castella, and Goudet (2007). We also used INBREEDR to calculate the 95% confidence interval of g_2 by bootstrapping over individuals and to permute the genetic data to generate a *p*-value for the null hypothesis of no variance in inbreeding in the sample (i.e., $g_2 = 0$) as described in detail by Stoffel et al. (2016).

2.7 | Testing for parentage assignment biases in our pedigree

The majority of accepted parental relationships had very high confidence (89% at ≥99% confidence). Nevertheless, Wang (2010) showed that parentage analyses can be biased in favour of heterozygotes, which could potentially create an artefactual positive relationship between sMLH and reproductive success. We evaluated whether such a bias could affect our pedigree by testing for an association between parental heterozygosity and the confidence with which parents were assigned in our pedigree using a generalized linear model (GLM) with a binomial error structure. A slight but statistically significant bias was found in the direction of homozygotes being assigned parentage with slightly greater confidence than heterozygotes (Table S1). To explore this further, we simulated pedigrees based on the empirical allele frequencies of our study population. Our methods and results are described in detail in the supplementary information. Briefly, initial simulations assuming random mating assigned 94% of parents with a probability of 1.0 and therefore no bias could be detected. Hence, we simulated an arguably more realistic pedigree with close inbreeding for which parentage analysis should be technically more challenging due to high relatedness among the candidate parents. Consistent with results from our empirical data set, we found that homozygotes had a slightly higher probability of being assigned parentage (Table S2). Taken together, these findings suggest that any bias in our pedigree should be both small and in the opposite direction to that predicted, and is therefore unlikely to generate a false signal of inbreeding depression.

2.8 | Statistical analyses

Strong inbreeding depression early in life will tend to deplete the adult population of inbred individuals and thereby reduce the power to detect inbreeding effects later in life (Huisman et al., 2016). To evaluate this possibility, we grouped individuals into six cohorts based on their survival to a given age (<1, 1, 2, 3, 4 or ≥5 years old) and used Levene's test to assess the equality of variances of f_{ped} and sMLH among the cohorts and Spearman's rank to test for a decrease in mean inbreeding with increasing age. We then investigated inbreeding depression for four main fitness components: (i) survival to nutritional independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv) annual reproductive success (see below for further details). These fitness components were used as response variables in four separate analyses conducted within R version 3.2.3 (R Core Team, 2014). Beforehand, all of the explanatory variables were checked for collinearity using pair plots and by calculating pairwise correlation coefficients. Graham (2003) showed that correlations between explanatory variables as low as 0.28 may compromise model parameterization but collinearity in our models was well below this, except for f_{ped} and sMLH, which we dealt with as described below. All of our models were also validated though visual inspection of histograms of residuals and plots of residuals against fitted values for each of the explanatory variables as recommend by Zuur, Leno, and Saveliev (2009).

For each analysis, we constructed a set of competing models, each incorporating prior knowledge of the banded mongoose system, and quantified their relative support using AIC_c weights within a multimodel inference framework. As support for a model increases, its AIC_c weight tends towards 1. To quantify the contributions of individual predictor variables, we then calculated predictor AIC_c weights by summing the AIC_c weights of all models containing that predictor. We also followed the recommendation of Richards, Whittingham, and Stephens (2011) and discarded models with better supported models nested within them (i.e., models that are more complicated versions of a better supported model).

Within the above framework, f_{ped} and sMLH were used as predictor variables to quantify the effects of inbreeding on fitness. Including f_{ped} and sMLH in the same models is likely to cause problems due to multicollinearity because both are estimates of IBD_g. Therefore, we quantified any potential effects of sMLH above and beyond f_{ped} by constructing a set of models containing both f_{ped} and the residuals of sMLH on f_{ped} (henceforth termed residual sMLH). As there is no statistical collinearity between f_{ped} and residual sMLH, we were able to include information from the pedigree and molecular markers simultaneously without biasing the regression parameter estimates (Graham, 2003). Residual sMLH can be interpreted as whether an individual is more or less heterozygous than expected given their f_{ped} and its effect size can be interpreted as its effect additional to that already made through its relationship with f_{ped} as any variance explained by both terms is attributed to f_{ped}. This technique is called sequential regression and performs well across a range of complex functional relationships and collinearity structures (Dormann et al., 2013). Additional nongenetic explanatory variables were analysed based on prior knowledge of the mongoose system as described below.

2.8.1 | Survival to nutritional independence

As mortality is highest in banded mongooses prior to nutritional independence around day 90, we first analysed survival to 90 days. A recent study found that offspring of extra group matings, which tend to be more heterozygous, have higher survivorship to 90 days (Nichols, Cant, & Sanderson, 2015), suggesting that there could be a direct link between inbreeding and early survivorship. In this study, data were available for a total of 489 individuals with all four grandparents assigned. Survival was analysed as a binomial response variable (coded as 1 = survived, 0 = died) within generalized linear mixed models (GLMMs) using LME4 (Bates, Maechler, Bolker, & Walker, 2015) with litter nested within pack as random effects. A total of 19 competing models were constructed (see Table 1), each containing different combinations of predictor variables representing plausible hypotheses to be evaluated within a multimodel inference framework. We included rainfall during the 30 days prior to birth as a predictor variable in all of the models, as this is robustly associated with early life survival (Nichols et al., 2015; Sanderson et al., 2015).

TABLE 1 Alternative models of survival to nutritional independence ranked in order of their AIC_c support

Model	Structure	*k*	Log likelihood	AIC_c	ΔAIC_c	AIC_c weight
M5	Rain + escorting	5	−271.954	554.033	0.000	0.348
M7	Rain + escorting + sMLH	6	−271.944	556.061	2.029	0.126
M6	Rain + escorting + f_{ped}	6	−271.953	556.081	2.048	0.125
M1	Rain	4	−274.286	556.655	2.623	0.094
M15	Rain * sMLH + escorting	7	−271.866	557.965	3.932	0.049
M11	Rain * f_{ped} + escorting	7	−271.917	558.066	4.034	0.046
M8	Rain + escorting + f_{ped} + residual sMLH	7	−271.939	558.110	4.078	0.045
M3	Rain + sMLH	5	−274.263	558.651	4.618	0.035
M2	Rain + f_{ped}	5	−274.282	558.688	4.655	0.034
M16	Rain * residual sMLH + escorting + f_{ped}	8	−271.811	559.923	5.890	0.018
M12	Rain * f_{ped} + escorting + residual sMLH	8	−271.902	560.103	6.071	0.017
M13	Rain * sMLH	6	−274.182	560.539	6.506	0.013
M9	Rain * f_{ped}	6	−274.203	560.580	6.547	0.013
M4	Rain + f_{ped} + residual sMLH	6	−274.248	560.669	6.637	0.013
M18	Rain * (f_{ped} + residual sMLH) + escorting	9	−271.781	561.937	7.905	0.007
M19	(Intercept only)	3	−278.019	562.087	8.054	0.006
M14	Rain * residual sMLH + f_{ped}	7	−274.091	562.415	8.382	0.005
M10	Rain * f_{ped} + residual sMLH	7	−274.168	562.568	8.536	0.005
M17	Rain * (f_{ped} + residual sMLH)	8	−274.022	564.345	10.312	0.002

See Section 2 for further details.

As escorting has a highly significant effect on survival to 60 days (Gilchrist, 2004) but is only weakly associated with survival to 90 days (Hodge, 2005), we also included escorting as a continuous variable (see above) in a subset of the models. To further test for an interaction between inbreeding and stress, we constructed a further subset of models containing interactions between rainfall and one of the inbreeding terms (i.e., rain * f_{ped} or rain * sMLH). As explained above, the effect of residual heterozygosity was evaluated by constructing models containing both f_{ped} and residual sMLH.

2.8.2 | Survival beyond nutritional independence

We investigated inbreeding depression for longevity based on all individuals that survived beyond 90 days (n = 428 mongooses with at least all four grandparents in the pedigree). Lifespan was investigated using Cox proportional hazard models in the SURVIVAL package (Therneau & Grambsch, 2000). Individuals that survived until the end of the study or that emigrated from the study population were classified as right censored in the models. To account for the nonindependence of individuals within social groups, we fitted pack as a frailty term, equivalent to a random effect. We also verified that the proportional hazard was independent of time using plots of the scaled Schoenfeld residuals. We constructed 14 competing models (see Table 2), all of which contained sex (coded as female = 0, male = 1) because males tend to have a longer lifespan (Cant, Nichols, Thompson, & Vitikainen, 2016). We used mean monthly rainfall in the first year of life as a predictor variable in a subset of models because it is associated with prey abundance and thereby influences lifespan (Marshall et al., 2017). As described above for the models of survival to nutritional independence, we also tested for an interaction between inbreeding and stress by constructing models containing interactions between rainfall and the inbreeding terms.

2.8.3 | Yearling body mass

We next investigated inbreeding depression for body mass (measured in g) at 1 year of age. Heavier banded mongoose females breed earlier (Hodge, 2005) and may thus have higher lifetime reproductive success. Also, yearling body mass exhibits inbreeding depression (Sanderson et al., 2015) although the study in question did not analyse microsatellite heterozygosity. Individuals were habituated to step onto a portable weighing balance for a small reward of milk, which allowed us to measure body mass. Yearling body mass was calculated as the average of all morning mass measurements for an individual taken between 350 and 380 days of age. Measurements were taken in the morning to standardize against fluctuations in body mass that may occur during the day. Data on yearling body mass were available for a total of 156 individuals with all four grandparents known. We constructed 53 competing models (See Table 3) with litter nested within pack as random effects. These models were run in the GLMMADMB package (Fournier, Skaug, Ancheta, & Ianelli, 2012) with a Gaussian error distribution. We included sex in a subset of models and rainfall in the 30 days prior to birth in a subset of the models as this was previously found to be positively associated

TABLE 2 Alternative models of survival beyond nutritional independence ranked in order of their AIC_c support

Model	Structure	*k*	Log likelihood	AIC_c	ΔAIC_c	AIC_c weight
M7	Sex + rain + sMLH	8.5	−1,645.576	3,297.209	0.000	0.261
M11	Sex + rain * sMLH	9.4	−1,644.911	3,297.916	0.707	0.183
M1	Sex	6.9	−1,647.964	3,297.938	0.728	0.181
M3	Sex + sMLH	8.1	−1,647.174	3,298.376	1.167	0.145
M5	Sex + rain	6.3	−1,647.560	3,299.149	1.939	0.099
M8	Sex + rain + f_{ped} + residual sMLH	7.9	−1,646.837	3,301.768	4.559	0.027
M2	Sex + f_{ped}	6.6	−1,649.023	3,302.074	4.865	0.023
M4	Sex + f_{ped} + residual sMLH	7.8	−1,648.015	3,302.086	4.876	0.023
M6	Sex + rain + f_{ped}	6.6	−1,648.164	3,302.385	5.176	0.020
M12	Sex + rain * residual sMLH + f_{ped}	8.6	−1,646.418	3,302.979	5.769	0.015
M10	Sex + rain * f_{ped} + residual sMLH	9.0	−1,646.708	3,303.559	6.350	0.011
M9	Sex + rain * f_{ped}	7.7	−1,648.083	3,304.261	7.052	0.008
M13	Sex + rain * (f_{ped} + residual sMLH)	9.7	−1,646.283	3,304.765	7.555	0.006
M14	(Intercept only)	4.9	−1,650.698	3,322.777	25.568	0.000

See Section 2 for further details.

TABLE 3 Alternative models of yearling body mass ranked in order of their AIC_c support

Model	Structure	*k*	Log likelihood	AIC_c	ΔAIC_c	AIC_c weight
M28	Sex + f_{ped}	6	−930.982	1,874.551	0.000	0.325
M32	Sex + rain + f_{ped}	7	−930.896	1,876.581	2.029	0.118
M36	Sex + index + f_{ped}	7	−930.935	1,876.659	2.107	0.113
M30	Sex + f_{ped} + residual sMLH	7	−930.955	1,876.699	2.147	0.111
M43	Sex + rain * f_{ped}	8	−930.509	1,878.039	3.488	0.057
M34	Sex + rain + f_{ped} + residual sMLH	8	−930.849	1,878.719	4.168	0.040
M40	Sex + rain + index + f_{ped}	8	−930.849	1,878.719	4.168	0.040
M38	Sex + index + f_{ped} + residual sMLH	8	−930.910	1,878.841	4.290	0.038
M44	Sex + rain * f_{ped} + residual sMLH	9	−930.436	1,880.158	5.606	0.020
M45	Sex + escorting + rain * f_{ped}	9	−930.448	1,880.182	5.630	0.019
M48	Sex + rain * residual sMLH + f_{ped}	9	−930.644	1,880.574	6.022	0.016
M42	Sex + rain + index + f_{ped} + residual sMLH	9	−930.804	1,880.894	6.342	0.014
M27	Sex	5	−935.417	1,881.251	6.699	0.011
M2	f_{ped}	5	−935.495	1,881.407	6.855	0.011

See Section 2 for further details. Only models with AIC_c weights greater than 0.01 are shown.

with body mass in one study (Nichols et al., 2015) but not in another (Sanderson et al., 2015). To test for interactions between inbreeding and stress, some of these models also included interactions between rainfall and the inbreeding terms. Escorting was included in a further subset of models as it correlates positively with pup weight at 84 days (Hodge, 2005; but see Gilchrist, 2004).

2.8.4 | Annual reproductive success

Reproductive success is closely linked to fitness, but no studies of banded mongooses have previously investigated inbreeding depression for this trait. We therefore used the pedigree to quantify annual reproductive success, expressed as the number of pups assigned to each individual, for all animals over 6 months of age who survived a given year. Because reproductive opportunities differ between the sexes, with most females breeding regularly while male reproductive success is strongly skewed towards the oldest three to five males in a pack (Nichols, Amos, Cant, Bell, & Hodge, 2010), separate models were constructed for each sex. These were based on a total of 240 annual observations of 99 females and 354 annual observations of 129 males. Annual reproductive success was modelled using a negative binomial error distribution with zero inflation within the R package GLMMADMB (Skaug, Fournier, Nielsen, & Magnusson, 2013). To account for multiple observations of individuals and packs, we fitted

individual and pack as random effects. We constructed 14 competing models separately for females and males (see Table 4a,b, respectively). As reproductive success tends to increase with age before tailing off later in life (Sanderson et al., 2015), we included age and age^2 as predictor variables in all of the models. Average monthly rainfall over the year was also included in a subset of models as a proxy for environmental stress, while inbreeding–stress interactions were investigated through the inclusion of models containing interactions between rainfall and the inbreeding terms.

3 | RESULTS

We augmented an existing microsatellite data set comprising 1,748 individuals genotyped at 35–43 microsatellite loci (Sanderson et al., 2015) by genotyping an additional 192 individuals at 35 microsatellites. This allowed us to enlarge the nine-generation deep banded mongoose pedigree of Sanderson et al. (2015) by increasing the number of maternal links from 1,570 to 1,725 and the number of paternal links from 1,476 to 1,625. The restricted data set of individuals with all four grandparents assigned, which formed the basis of all subsequent analyses, increased from 672 to 777.

3.1 | Inbreeding and heterozygosity

Our pedigree uncovered appreciable variance in inbreeding (mean f_{ped} = 0.058, variance = 0.006), with the majority of individuals (66.4%) being to some extent inbred (Figure 1, top marginal histogram). Weak inbreeding ($0 < f_{ped} < 0.125$) accounted for 46.5% of the population, while 12.9% of individuals were moderately inbred ($0.125 \leq f_{ped} < 0.25$) and 7.1% were closely inbred ($f_{ped} \geq 0.25$). Microsatellite heterozygosity (sMLH) was approximately normally distributed with a mean of 0.982 and a variance of 0.034 (Figure 1, right marginal histogram) and correlated significantly with f_{ped} ($R = -.34$, $p < .001$). Furthermore, the measure g_2, which quantifies the extent to which heterozygosity is correlated across loci, was positive (0.012, 95% CI = 0.007–0.018) indicating that the microsatellites are capturing variation in inbreeding. As observed in other species (e.g., Huisman et al., 2016), appreciable variation was observed in sMLH among individuals with the same f_{ped}.

TABLE 4 Alternative models of annual reproductive success in (a) females, and (b) males, ranked in order of their AIC$_c$ support

Model	Structure	*k*	Log likelihood	AIC$_c$	ΔAIC$_c$	AIC$_c$ weight
(a)						
M4	Age + age^2 + f_{ped}	8	−329.679	675.981	0.000	0.286
M1	Age + age^2	7	−330.848	676.179	0.197	0.259
M5	Age + age^2 + rain + f_{ped}	9	−329.642	678.067	2.085	0.101
M8	Age + age^2 + f_{ped} + residual sMLH	9	−329.652	678.087	2.105	0.100
M3	Age + age^2 + sMLH	8	−330.790	678.203	2.222	0.094
M2	Age + age^2 + rain	8	−330.808	678.239	2.258	0.092
M7	Age + age^2 + rain + f_{ped} + residual sMLH	10	−329.625	680.211	4.229	0.034
M6	Age + age^2 + rain + sMLH	9	−330.733	680.249	4.267	0.034
M14	(Intercept only)	5	−369.474	749.204	73.223	0.000
(b)						
M8	Age + age^2 + f_{ped} + residual sMLH	9	−300.139	618.801	0.000	0.494
M7	Age + age^2 + rain + f_{ped} + residual sMLH	10	−300.133	620.907	2.106	0.172
M12	Age + age^2 + rain * residual sMLH + f_{ped}	11	−299.697	622.166	3.365	0.092
M10	Age + age^2 + rain * f_{ped} + residual sMLH	11	−300.051	622.874	4.073	0.065
M3	Age + age^2 + sMLH	8	−303.333	623.083	4.282	0.058
M4	Age + age^2 + f_{ped}	8	−303.792	624.001	5.200	0.037
M13	Age + age^2 + rain * (f_{ped} + residual sMLH)	12	−299.663	624.241	5.440	0.033
M6	Age + age^2 + rain + sMLH	9	−303.332	625.187	6.386	0.020
M5	Age + age^2 + rain + f_{ped}	9	−303.779	626.081	7.280	0.013
M11	Age + age^2 + rain * smlh	10	−302.895	626.431	7.630	0.011
M9	Age + age^2 + rain * f_{ped}	10	−303.725	628.091	9.290	0.005
M1	Age + age^2	7	−309.393	633.110	14.308	0.000
M2	Age + age^2 + rain	8	−309.390	635.197	16.396	0.000
M14	(Intercept only)	5	−343.651	697.474	78.673	0.000

The models of female annual reproductive success which included inbreeding–stress interactions failed to converge and so were omitted. See Section 2 for further details.

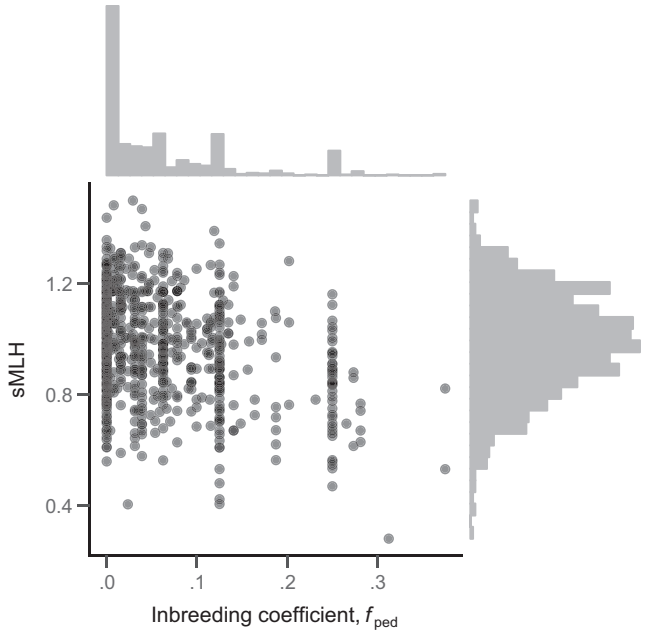

FIGURE 1 The relationship between the pedigree-based inbreeding coefficient, f_{ped} and sMLH for 777 banded mongoose individuals with all four grandparents assigned (R = .34, p < .001). Scatter on the y-axis for a given f_{ped} value represents variation in microsatellite heterozygosity among individuals with the same pedigree inbreeding coefficient. Marginal histograms show the distributions of f_{ped} (top) and sMLH (right axis)

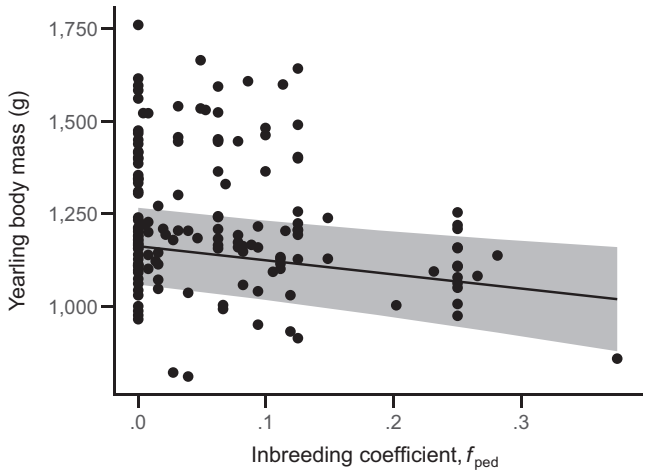

FIGURE 2 The relationship between f_{ped} and yearling body mass. The trend line shows the expected body mass of a female yearling, and the shaded region shows the 95% confidence interval

3.2 | Changes in inbreeding with age

If inbred individuals experience stronger viability selection early in life, the variance in inbreeding should be lower in adults, making it more difficult to detect inbreeding depression for late-acting traits (Huisman et al., 2016). To investigate this possibility, we divided the mongooses into six cohorts based on their survival to a given age (see Section 2.8) and tested for differences in the variance of f_{ped} and sMLH among these cohorts using Levene's tests. Neither of the inbreeding measures showed a decrease in variance with age (Table S3) and the variance in sMLH did not differ significantly among cohorts (F_5 = 0.74, p = .59). However, the cohorts did not have equal variance in f_{ped} (F_5 = 2.36, p = .03). This result appears to be driven by low sampling variance in individuals who survived between 1 and 2 years as the variance in f_{ped} no longer differed significantly among cohorts after these animals were excluded from the analysis. Taken together, these findings suggest that viability selection against inbred individuals does not reduce the variance in inbreeding with age. In line with this, we also found no evidence for a decline in the mean level of inbreeding with increasing age (f_{ped} ρ = 0.043, p = .23; sMLH ρ = −0.01, p = .78; Table S3).

3.3 | Survival to nutritional independence

We found that the model of survival to nutritional independence with the greatest AIC_c support included rainfall in the 30 days prior to birth and escorting as fixed effect explanatory variables (Table 1, intercept = −0.5364 ± 0.4514 *SE*, rainfall β = 0.3577 ± 0.1348 *SE*, escorting β = 0.8764 ± 0.4084 *SE*, random effects: pack *SD* = 0.000, litter nested within pack *SD* = 1.57). The second and third most supported models included rain and escorting as well as an inbreeding term (Table 1). However, as they had the best model nested within them (i.e., they were more complex but less supported versions of the first model), we did not consider them further, as recommended by Richards et al. (2011).

3.4 | Survival beyond nutritional independence

The results of our analysis of adult survival were equivocal (Table 2). The highest ranking model included sMLH but had roughly equivalent AIC_c support (ΔAIC_c < 1) to a simple model that included only sex. As AIC_c tends to slightly favour complex models, especially when there is uncertainty over the best model (Symonds & Moussalli, 2011), our results do not provide convincing evidence of inbreeding depression for longevity.

3.5 | Yearling body mass

By contrast, strong support was found for inbreeding depression in yearling body mass, with all of the top 12 models containing f_{ped} as a fixed effect explanatory variable (Table 3) and the predictor AIC_c weight for f_{ped} being high at 0.96. The top ranking model contained sex and f_{ped} (Table 3, Figure 2; intercept = 1162 ± 53 *SE*, sex β = 59 ± 19 *SE* f_{ped} β = −382 ± 127 *SE*, random effects: pack *SD* = 125.5, litter nested within pack *SD* = 37.6). As before, we disregarded less supported models with this model nested within them as suggested by Richards et al. (2011).

3.6 | Annual reproductive success

Focusing first on female reproductive success, the top ranking model contained age + age^2 + f_{ped} but the next best model had very similar

AIC_c support but did not contain f_{ped} (Table 4a). Because AIC_c support for these two models was so similar and AIC exhibits a slight preference for overly complex models, the simpler model should be preferred. Consequently, our data provided only limited support for inbreeding depression for female annual reproductive success as our preferred model contained only age and age^2 (intercept = −1.2539 ± 0.3773 *SE*, age β = 0.7616 ± 0.1776 *SE*, age^2 β = −0.0480 ± 0.0244 *SE*). By contrast, the best supported model for males contained both f_{ped} and residual sMLH (intercept = −2.9481 ± 0.4792 *SE*, age β = 1.4452 ± 0.1905 *SE*, age^2 β = −0.1343 ± 0.0209 *SE*, f_{ped} β = −6.2994 ± 1.7203 *SE*, residual sMLH β = 2.0920 ± 0.7646 *SE*). This not only provides evidence for inbreeding depression for male annual reproductive success, but also suggests that marker heterozygosity captures a significant amount of variance that is not explained by f_{ped}. This model was nested within the second and third highest ranking models, which also had considerable AIC_c support and respectively contained rain and an interaction between rain and f_{ped}.

Consistent with theoretical expectations, the best supported model of annual male reproductive success revealed a negative association with f_{ped} (Figure 3a) and a positive association with residual sMLH (Figure 3b). Inbred males with an f_{ped} value of 0.25 were predicted by the model to have approximately 79% fewer offspring than fully outbred individuals with an f_{ped} value of zero, while males with residual sMLH values one standard deviation above zero (0.185) were predicted to have 47% more offspring than individuals with residual sMLH equal to zero. This indicates that within f_{ped} classes, relatively heterozygous individuals tend to have greater reproductive success.

3.7 | Effect sizes of the inbreeding terms

To provide further insights into the effect sizes of the inbreeding terms, we constructed three alternative models separately for each fitness trait. These models contained noninbreeding terms that were retained in the top ranking models described above for each trait, while in addition, the first model contained f_{ped}, the second contained sMLH, and the third contained f_{ped} plus residual sMLH. To evaluate inbreeding effects, we then calculated effect sizes and their corresponding 95% confidence intervals (CIs) for all of the predictor variables contained in each model. The results are summarized separately for each trait in Figure 4. Consistent with results from the information theoretic approach, the 95% CIs of the effect sizes of all three inbreeding terms overlapped zero for survival to nutritional independence, survival beyond nutritional independence and female reproductive success (Figure 4a,b,d), suggesting that there is very little evidence for inbreeding depression for these traits. Also as expected, f_{ped} had negative point estimates whose corresponding 95% CIs did not overlap zero in models of yearling body mass and annual male reproductive success (Figure 4c,e), while sMLH and residual sMLH only had positive estimates and 95% CIs not overlapping zero in models of male reproductive success (Figure 4e).

3.8 | Associated *p*- and R^2 values

In order to evaluate the sensitivity of our results to the statistical framework employed, we determined the statistical significance of f_{ped}, sMLH and residual sMLH using a frequentist approach. Separately for each trait, we derived *p*-values for each of the inbreeding terms using likelihood ratio tests. The significance of f_{ped} and sMLH was derived by comparing models containing these terms with equivalent "null models" containing only the relevant noninbreeding terms, while *p*-values for residual sMLH were obtained through the comparison of models containing f_{ped} plus residual sMLH with equivalent models containing only f_{ped}. To provide an indication of the proportion of variance explained by each model, we also calculated conditional R^2 values for GLMMs (Nakagawa & Schielzeth, 2013) and Cox and Snell's pseudo R^2 values for Cox proportional hazard models (Cox & Snell, 1989). However, this was not possible for zero-inflated negative binomial GLMMs so we instead report log likelihood values for these models (Table 5). To allow direct comparison with other studies, correlation coefficients between the two inbreeding measures and each fitness trait are also provided in the supporting information (Table S4). Consistent with the results of the multimodel approach described above, we found a highly

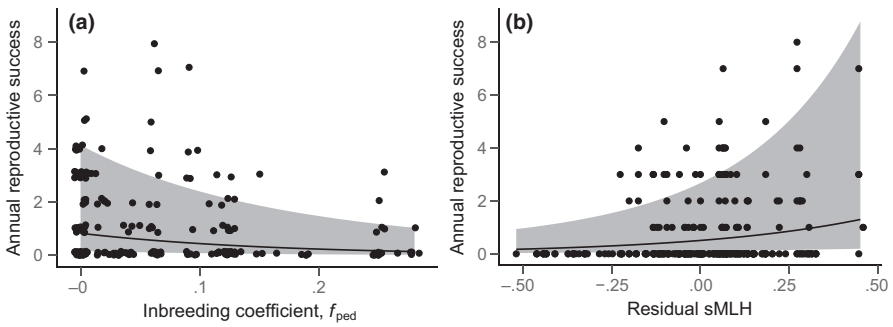

FIGURE 3 The relationship between annual male reproductive success and (a) f_{ped}, and (b) residual sMLH derived from a single model (M8 in Table 4b) where both inbreeding measures were fitted together. The trend lines show expected values based on average age, and the shaded regions show associated 95% confidence intervals. Data points in plot (a) were given a small amount of jitter to avoid over plotting

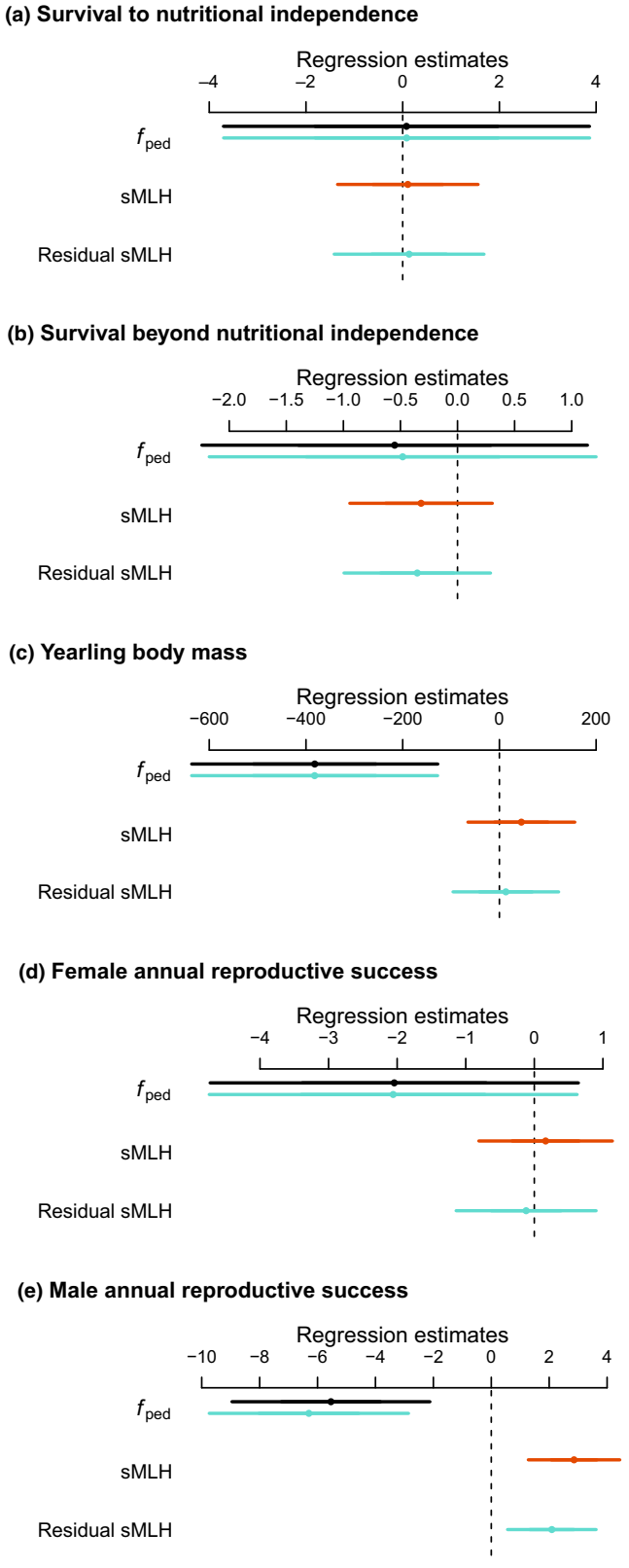

FIGURE 4 Estimated regression coefficients of the three inbreeding terms in models of five different fitness traits, showing point estimates and associated 95% confidence intervals. Each panel shows three different models–one containing f_{ped} (shown in black), one containing sMLH (shown in dark orange), and one containing f_{ped} + residual sMLH (shown in light turquoise) as described in the Results section. In addition to these inbreeding terms, all of the models contained other fixed effects, but these are not shown for ease of interpretation. The larger confidence intervals of f_{ped} relative to sMLH result from its smaller range (Figure 1) [Colour figure can be viewed at wileyonlinelibrary.com]

significant effect of f_{ped} on yearling body mass, which explained almost 5% of the total variation (Table 5c), although sMLH did not explain a significant amount of variance in this trait. By contrast, both f_{ped} and sMLH explained significant variation in male annual reproductive success (Table 5e). Furthermore, adding residual sMLH to a model containing only f_{ped} resulted in a significant improvement to the model of annual male reproductive success (p = .007, Table 5e), suggesting that for some traits, genetic markers may capture variation in inbreeding above and beyond that explained by f_{ped}.

3.9 | Sensitivity to marker number

To further investigate the explanatory power of f_{ped} and marker heterozygosity, we directly compared three of our models of annual male reproductive success in which the inbreeding terms were f_{ped} (M4 in Table 4b), sMLH (M3 in Table 4b) and f_{ped} plus residual sMLH (M8 in Table 4b) respectively, and explored the sensitivity of model AIC_c to marker number. As expected, AIC_c decreased steadily with increasing marker number (Figure 5). With fewer than around 20 markers, sMLH did not perform as well as f_{ped}, but with 30–40 markers, AIC_c values for the two models were very similar. Furthermore, the model containing both f_{ped} and residual sMLH became increasingly superior to the model containing only f_{ped} as more markers were deployed.

3.10 | Testing for local effects

Finally, we tested for the possible involvement of local effects involving specific microsatellite loci by adapting the approach of Szulkin, Bierne, and David (2010). Specifically, we compared a model of male reproductive success containing age, age^2, f_{ped} and residual sMLH with a model in which residual sMLH was replaced by separate terms for the residual heterozygosity of each of the microsatellite loci. The second model was not a significant improvement over the first, although the corresponding p-value was close to significance ($-2LL_{30}$ = 42.06, p = .07). Our results are therefore more consistent with inbreeding depression than with a mechanism based on one or a small number of local effects.

4 | DISCUSSION

Although inbreeding depression is known to be important in many wild populations, relatively few studies are large and detailed enough either to compare multiple traits at different stages in the life cycle or to investigate the relative explanatory power of pedigree-based

TABLE 5 Statistical significance and variance explained by inbreeding terms in models of five fitness traits

(a) Survival to nutritional independence			
Binomial GLMM, n = 489			
Structure	**Likelihood ratio**	***p*-value**	**Conditional R^2_{glmm}**
Rain + escorting			.4701
Rain + escorting + f_{ped}	0.0017	.9671	.4702
Rain + escorting + sMLH	0.0213	.8839	.4703
(b) Survival beyond nutritional independence			
Cox proportional hazard model, n = 428			
Structure	**Likelihood ratio**	***p*-value**	**Cox and Snell's pseudo R^2**
Sex			.0817
Sex + f_{ped}	2.1178	.1456	.0755
Sex + sMLH	1.5803	.2087	.0863
(c) Yearling body mass			
Gaussian GLMM, n = 150			
Structure	**Likelihood ratio**	***p*-value**	**Conditional R^2_{glmm}**
Sex			.5734
Sex + f_{ped}	8.87	.0029	.6221
Sex + sMLH	0.674	.4117	.5766
(d) Female annual reproductive success			
Zero-inflated, negative binomial GLMM, n = 240			
Structure	**Likelihood ratio**	***p*-value**	**Log likelihood**
Age + age^2			−330.848
Age + age^2 + f_{ped}	2.338	.1263	−329.679
Age + age^2 + sMLH	0.116	.7334	−330.790
(e) Male annual reproductive success			
Zero-inflated, negative binomial GLMM, n = 354			
Structure	**Likelihood ratio**	***p*-value**	**Log likelihood**
Age + age^2			−309.393
Age + age^2 + f_{ped}	11.202	.0008	−303.792
Age + age^2 + sMLH	12.12	.0005	−303.333
Age + age^2 + f_{ped} + residual sMLH	7.306	.0069	−300.139

The significance of f_{ped} and sMLH was derived by comparing models containing these terms with equivalent "null models" containing only the relevant noninbreeding terms, while *p*-values for residual sMLH were obtained through the comparison of models containing f_{ped} + residual sMLH with equivalent models containing only f_{ped}. For each trait, the models that we constructed are listed in the first column of the table, with the null model shown first. Conditional R^2_{glmm} was calculated following Nakagawa and Schielzeth (2013) and Cox and Snells's pseudo R^2 was calculated using the number of uncensored observations rather than the total number of observations as recommended by O'Quigley, Xu, and Stare (2005). As R^2 values cannot be calculated for zero-inflated negative binomial GLMMs, log likelihood values are presented as a measure of the fit of models of annual male reproductive success.

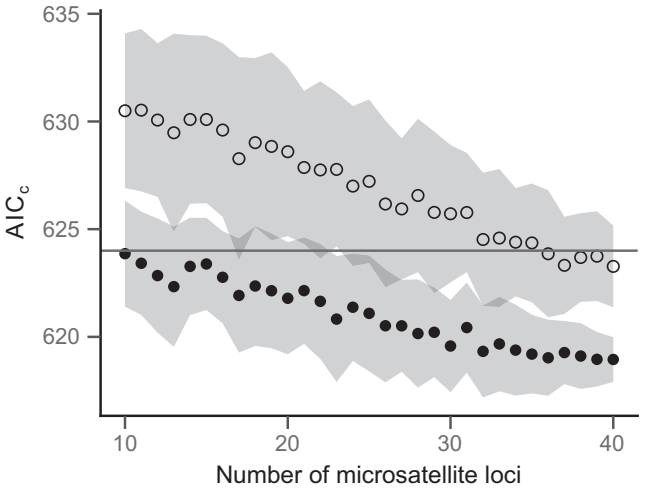

FIGURE 5 The relationship between AIC$_c$ of models of annual male reproductive success and the number of microsatellites used to calculate standardized multilocus heterozygosity. Open points represent models with the structure: age + age^2 + sMLH; closed points represent models with the structure: age + age^2 + f_{ped} + residual sMLH. The horizontal line represents a model with the structure: age + age^2 + f_{ped}. We selected n different microsatellite loci at random and calculated heterozygosity as sMLH 100 times for each value of n. Points represent mean values, and the shaded regions indicate ±1 *SD*

and molecular estimates of inbreeding. We therefore used an exceptionally comprehensive long-term study of banded mongooses both to quantify inbreeding depression for early- and late-acting traits and to evaluate the hypothesis that marker heterozygosity may capture fitness variation above and beyond that explained by f_{ped}. Contrary to our initial expectations, we did not find evidence for strong viability selection against inbred individuals early in life, but instead detected inbreeding depression for traits relating to individual quality (i.e., yearling body mass and male annual reproductive success). Furthermore, we found that fitting f_{ped} and residual sMLH together in a single model explained significantly more of the variance in male annual reproductive success than using f_{ped} alone. However, this was not the case for yearling body mass, where f_{ped} explained variation in fitness but sMLH did not.

4.1 | Inbreeding depression for different traits

Theory predicts that inbreeding depression should be greatest for traits closely linked to fitness because traits under strong directional selection will exhibit greater directional dominance (Lynch & Walsh, 1998). This is supported by a meta-analysis that found stronger inbreeding depression for life-history traits such as survival and fecundity than for morphological traits such as body weight (DeRose & Roff, 1999). Given that all of the traits we analysed in banded mongooses are arguably very closely linked to fitness, we were

initially surprised not to find inbreeding depression for either survival to nutritional independence or longevity. One potential explanation for this is that inbreeding depression for early survival could be buffered by the social system of this species (Ihle, Hutter, & Tschirren, 2017; Nielsen et al., 2012; Pilakouta, Jamieson, Moorad, & Smiseth, 2015) especially if escorts preferentially direct care towards inbred individuals (Thünken, Bakker, Baldauf, & Kullmann, 2007). However, due to the complexity of the banded mongoose system, testing this hypothesis lies beyond the scope of the current study. Alternatively, as the environment is relatively benign and major causes of death in our study population are predation and injuries sustained during aggressive interactions between social groups (Cant et al., 2013), there may be relatively little scope for strong genetic effects on survival. A further possibility is that our study may have lacked the statistical power to detect inbreeding depression for traits with smaller available sample sizes, such as female annual reproductive success. However, this seems unlikely to account for the absence of detectable inbreeding depression for early-acting traits like survival to nutritional independence as sample sizes for these analyses were more than double what was available for yearling body mass, where inbreeding depression was detected. Nevertheless, we cannot discount the possibility that inbreeding depression might influence survival at an even earlier stage of development, for instance *in utero* or during their first month *post partum* before emergence from the underground den.

As several studies have shown that inbreeding depression can be magnified by stress (Armbruster & Reed, 2005; Fox & Reed, 2011; Meagher, Penn, & Potts, 2000; Norén, Godoy, Dalén, Meijer, & Angerbjörn, 2016; Reed, Fox, Enders, & Kristensen, 2012), we included interactions between rainfall and both measures of inbreeding in all of our analyses as rainfall is a proxy for food availability. We found that none of the top ranking models of survival to nutritional independence, longevity, yearling body mass or annual reproductive success contained interactions between rainfall and either f_{ped} or sMLH. Furthermore, although rainfall has a strong effect on survival to nutritional independence (Nichols et al., 2015; Sanderson et al., 2015) and was therefore included as a main effect in all models of this particular trait, rainfall did not feature in any of the chosen models of the other three fitness traits. Thus, our rainfall measures do not appear to strongly influence most of the investigated traits, which may help to explain why interactions involving rainfall were not found.

Alternatively, social stressors might be disproportionately important in this cooperative breeding species. Consistent with this, strong inbreeding depression was found for male annual reproductive success, with closely inbred individuals ($f_{ped} \geq 0.25$) having 79% lower annual reproductive success than individuals with an f_{ped} of zero, whereas our results for female reproductive success provided at best limited support for inbreeding depression. Although the sample size of female observations was smaller, sex-specific inbreeding depression would be consistent with previous studies of wild mice showing that male–male competition amplifies inbreeding depression (Meagher et al., 2000). It would also be in line with stronger reproductive skew in male vs. female banded mongooses (Nichols et al., 2010) as stronger directional selection is expected to increase inbreeding depression.

4.2 | Detecting inbreeding depression with pedigrees and genetic markers

Pedigrees have for many years been the gold standard for quantifying inbreeding depression in wild populations (Pemberton, 2004, 2008). However, pedigree data are often incomplete and assignment errors can introduce significant error into the estimation of f_{ped} (Reid et al., 2014) while the assumption that the founders are outbred and unrelated to one another may also be violated in closed or structured populations. In addition, f_{ped} is a measure of the expected IBD_g of an individual based on its pedigree and cannot capture stochastic variation in realized IBD_g resulting from Mendelian segregation (Hedrick et al., 2016; Hill & Weir, 2011; Knief et al., 2017). Consequently, there has been growing interest in the extent to which f_{ped} and marker heterozygosity can capture inbreeding effects, either independently or when analysed together, as well as in how the explanatory power of genetic markers varies with the number of loci that can be genotyped.

Several studies have compared the ability of pedigrees and microsatellites to detect inbreeding depression. These have reached the general consensus that f_{ped} usually performs better (e.g., Ólafsdóttir & Kristjánsson, 2008; Slate et al., 2004; Taylor et al., 2010), even when hundreds of microsatellites are used (Nietlisbach et al., 2017), although it is also to be expected that tens of thousands of SNPs will outperform f_{ped} (Huisman et al., 2016; Kardos et al., 2015). Nevertheless, both Forstmeier et al. (2012) and Hammerly et al. (2013) detected stronger inbreeding effects with around ten microsatellites than with f_{ped}. Our results fall somewhere in between these opposite ends of the spectrum, with heterozygosity based on around 40 microsatellites having roughly equivalent explanatory power to f_{ped} for male annual reproductive success but not for yearling body mass. This probably reflects a variety of factors as discussed below.

First, most pedigrees suffer to a greater or lesser extent from errors in the assignment of parental relationships, which can lead to significant and often downward bias in the estimation of inbreeding depression (Reid et al., 2014). This could partly explain the contrasting results of Nietlisbach et al. (2017) and Hammerly et al. (2013), as the former study was able to genotype the parents of all of the individuals used in the analysis for a very large number of microsatellites, resulting in an unusually accurate pedigree, whereas Hammerly et al. (2013) recognized that their pedigree contained a significant number of errors. Although it is difficult to directly compare different studies, our banded mongoose pedigree probably sits closer to the song sparrow end of the continuum, as our panel of microsatellites was moderately large and the majority of the adult population (all but four parents, Sanderson et al., 2015) was included.

A second factor that may influence the relative explanatory power of pedigrees and genetic markers is pedigree depth. Pedigree-

based inbreeding estimates become increasingly accurate with increasing depth, although these estimates become only marginally more precise beyond five generations in populations with certain structures (Kardos et al., 2015; Slate et al., 2004). Therefore, deeper pedigrees will tend to capture more of the variance in IBD_g within a given population and leave less "undetected inbreeding" for the markers to capture (Nietlisbach et al., 2017). This could potentially help to explain why residual heterozygosity accounts for additional fitness variation in one of the two traits that showed inbreeding depression in our study, as 54% of individuals in the song sparrow pedigree had eight or more known ancestral generations, whereas our equivalent value was only 3% and around half of all individuals in our banded mongoose pedigree had fewer than five generations known.

Third, the information content of the genetic markers used in a study will influence how well heterozygosity measures inbreeding. Homozygosity measured at genetic markers with few alleles and/or highly skewed allele frequencies is more likely by chance to reflect IBS than IBD and so may provide relatively little information about an individual's level of inbreeding. Calculating the IBD–IBS discrepancy for our data set following Knief et al. (2017) resulted in an estimate of 49%. This is higher than in zebra finches (13%, Knief et al., 2017) and may in part reflect the relatively low allelic richness of our microsatellites (average number of alleles = 5.2, Supplementary Table S5). However, this does not appear to have been a major issue for our study, probably due to the relatively large panel of available microsatellites. It might be interesting to explore this further in future studies by attempting to develop "ideal markers" where there is little to no IBD–IBS discrepancy. One possible strategy would be to genotype small panels of SNPs residing within known runs of homozygosity (ROH) following the suggestion of Knief et al. (2017).

In addition, factors intrinsic to a given system may also play a role, such as the frequency of close inbreeding, the number of chromosomes and genetic map length. For example, theoretical work by Hill and Weir (2011) and simulations by Hedrick et al. (2016) suggest that the variation in realized IBD_g around that expected by f_{ped} will be greater for closer inbreeding and hence that the type and variance of inbreeding in a population will affect how well f_{ped} estimates IBD_g. We know that close inbreeding is relatively common in banded mongooses, not because of small population sizes but because both sexes frequently remain in their natal group for their entire lives and breed with other group members (Nichols et al., 2014). Hence, the relatively high frequency of close inbreeding in this species could potentially help to explain our results.

Furthermore, f_{ped} will be relatively imprecise in species with fewer chromosomes and shorter genetic maps because genomes inherited in larger blocks will exhibit greater variance in realized IBD_g for a given value of f_{ped} (Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015; Stam, 1980). Genomes inherited in larger blocks should therefore provide greater scope to detect inbreeding depression with relatively few molecular markers (Forstmeier et al., 2012). The size of these blocks is partly determined by the number of chromosomes because the proportion of unlinked loci will increase with chromosome number (Weir, Avery, & Hill, 1980), while within chromosomes, both the number and distribution of crossovers will play a role (Knief et al., 2017). To illustrate this point, nearly a third of the zebra finch genome segregates in only four blocks because almost half of the autosomal genome comprises four chromosomes that experience very little recombination (Forstmeier et al., 2012). It is currently difficult for us to judge how these factors could have influenced our results as the number of chromosomes in banded mongoose is neither small nor large ($2n = 36$, Fredga, 1972) and the recombination landscape of this species has not yet been characterized.

Factors that influence the relative ability of f_{ped} and markers to detect inbreeding depression will also vary among populations and are expected to differ systematically between large populations and smaller, threatened ones. Small or fragmented populations often have higher rates of inbreeding and lower genetic diversity, and Grueber, Wallis, and Jamieson (2008) argue that these and other differences make it difficult to generalize results from outbred populations to threatened ones. It is therefore worth considering how similar systems are in the prevalence of inbreeding before extrapolating results between them. Furthermore, historical changes in the structure of a population, including bottlenecks and population admixture, may also create variance in inbreeding *sensu lato* (Bierne, Tsitrone, & David, 2000; Grueber et al., 2008; Weir et al., 1980). Consequently, the number of markers needed to accurately quantify IBD_g will also depend on the demographic history of the population in question (Miller et al., 2014).

4.3 | Capturing inbreeding depression with sequential regression

Although pedigrees clearly fail to capture variation in heterozygosity about the genomewide expectation given by f_{ped}, relatively few studies have attempted to quantify the amount of fitness variation that genetic markers might capture additional to that explained by f_{ped}. Some studies approached this question by fitting f_{ped} and heterozygosity as predictor variables in the same statistical models of the focal traits (e.g., Bensch et al., 2006; Grueber et al., 2011; Nietlisbach et al., 2017). However, this approach may be problematic because heterozygosity is often correlated with f_{ped} and including collinear variables in a model can lead to inaccurate parameter estimates (Graham, 2003). We therefore used sequential regression as an alternative approach that attributes all of the shared variance to f_{ped} and is therefore able to estimate how well marker heterozygosity explains variation in fitness after controlling for f_{ped} without biasing parameter estimates. Using an information theoretic approach, we found that the best model of male annual reproductive success contained residual sMLH as well as f_{ped}. This was also supported by a frequentist approach, which uncovered a highly significant ($p = .007$) effect of residual sMLH. By contrast, residual sMLH did not explain significant variation in yearling weight. One potential explanation for this could be that male reproductive success exhibits stronger inbreeding depression, which may make residual heterozygosity effects easier to detect.

An alternative to controlling statistically for f_{ped} is to control for this experimentally by screening genetic markers in individuals chosen to have the same f_{ped}. For example, Hemmings et al. (2012) used 384 genomewide distributed SNPs to estimate homozygosity in zebra finches with the same f_{ped}, finding that the most homozygous birds were less likely to survive to sexual maturity. This study echoes an earlier paper where full-sibling reed warblers were compared (Hansson, Bensch, Hasselquist, & Åkesson, 2001) and where again heterozygosity correlated with fitness despite identical f_{ped}. A key difference is that Hansson et al. (2001) used five microsatellites, leading the authors to conclude that a local effect was responsible, whereas the much larger panel used by Hemmings et al. (2012) more or less precludes a dominant role for only one or two loci. Consistent with the latter study, two lines of evidence are suggestive of a genomewide mechanism in banded mongooses. First, in our models of annual male reproductive success, we found that AIC_c steadily fell as the number of randomly sampled microsatellite loci increased, regardless of whether sMLH or residual sMLH were fitted as predictor variables. Second, we did not find that a model incorporating the single-locus heterozygosities of all of the loci explained significantly more variation than a model containing only sMLH. Although the second test is admittedly conservative, collectively our results point towards a polygenic architecture, consistent with the widespread view that the majority of inbreeding effects are caused by many loci with small effect sizes distributed across the genome (Charlesworth & Willis, 2009; Szulkin et al., 2010).

4.4 | Future perspectives

Looking to the future, although ours and many other studies have quantified heterozygosity using microsatellites, simulations clearly indicate that tens of thousands of markers will outperform even very deep pedigrees at capturing inbreeding depression, particularly when they can be mapped to a reference genome to quantify ROH (Kardos et al., 2015; Wang, 2016). This is supported by a growing number of empirical studies of wild populations using approaches like restriction site-associated DNA sequencing (Hoffman et al., 2014), high density SNP arrays (Chen et al., 2016; Huisman et al., 2016) and whole-genome resequencing (Kardos et al., 2018). As the costs of these and related methods continue to fall, they are likely to become preferred approaches for studying inbreeding and its consequences in wild populations.

5 | CONCLUSION

We used a high-quality pedigree together with data from up to 43 microsatellites to investigate inbreeding depression in a cooperatively breeding species where mating between close relatives is common. We detected inbreeding depression for yearling body weight and annual male reproductive success but found no evidence for inbreeding affecting survival, either to nutritional independence or beyond. Furthermore, for one of the two traits exhibiting inbreeding depression, our panel of microsatellites had similar explanatory power to f_{ped} and residual sMLH explained a significant proportion of fitness variation when fitted in a model together with f_{ped}. Our findings therefore suggest that, at least under some circumstances, combining pedigree and molecular measures of inbreeding may allow us to explain more fitness variation and thereby improve our understanding of the genetic variance underpinning fitness variation in wild populations.

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AUTHOR CONTRIBUTION

J.I.H. and H.J.N. designed the research; D.A.W. genotyped individuals and assigned parentage and sibships; D.A.W. conducted data analyses with assistance from J.I.H.; J.I.H. and D.A.W. wrote the manuscript with comments from H.J.N.; field data were collected by H.J.N. and M.A.C. All of the authors read and commented upon the final manuscript.

DATA ACCESSIBILITY

Microsatellite genotypes, pedigree inbreeding coefficients, and lifetime and annual data records are available via Dryad https://doi.org/10.5061/dryad.bq868sh. All of the computer code used to analyse the data are provided as R script files.

ORCID

David A. Wells http://orcid.org/0000-0002-4531-5968
Michael A. Cant http://orcid.org/0000-0002-1530-3077
Hazel J. Nichols http://orcid.org/0000-0002-4455-6065
Joseph I. Hoffman http://orcid.org/0000-0001-5895-8949

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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