Conserv Genet
DOI 10.1007/s10592-010-0147-z

RESEARCH ARTICLE

Inbreeding, heterozygosity and fitness in a reintroduced population of endangered African wild dogs (*Lycaon pictus*)

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Received: 22 February 2010 / Accepted: 24 September 2010
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Abstract It is crucial to understand the genetic health and implications of inbreeding in wildlife populations, especially of vulnerable species. Using extensive demographic and genetic data, we investigated the relationships among pedigree inbreeding coefficients, metrics of molecular heterozygosity and fitness for a large population of endangered African wild dogs (*Lycaon pictus*) in South Africa. Molecular metrics based on 19 microsatellite loci were significantly, but modestly correlated to inbreeding coefficients in this population. Inbred wild dogs with inbreeding coefficients of $\geq$0.25 and subordinate individuals had shorter lifespans than outbred and dominant contemporaries, suggesting some deleterious effects of inbreeding. However, this trend was confounded by pack-specific effects as many inbred individuals originated from a single large pack. Despite wild dogs being endangered and existing in small populations, findings within our sample population indicated that molecular metrics were not robust predictors in models of fitness based on breeding pack formation, dominance, reproductive success or lifespan of individuals. Nonetheless, our approach has generated a vital database for future comparative studies to examine these relationships over longer periods of time. Such detailed assessments are essential given knowledge that wild canids can be highly vulnerable to inbreeding effects over a few short generations.

Keywords Endangered · Heterozygosity-fitness correlation · Inbreeding · Lifespan · *Lycaon pictus*

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Introduction

As habitats shrink and fragment, inbreeding increasingly becomes a serious threat to local wildlife populations, often resulting in the loss of genetic diversity and inbreeding depression (Frankham et al. 2002). The consequences of increased homozygosity for individual fitness has been shown repeatedly within captive populations, leading to increased neonatal and juvenile mortality (Ralls et al. 1979) as well as compromised reproduction (Fredrickson et al. 2007) and longevity (Charpentier et al. 2008). Due to inherent logistical challenges, much less is known about

Published online: 10 October 2010

the influence of inbreeding within natural populations, an issue which now has become a major priority in conservation genetics (Frankham et al. 2002; Keller and Waller 2002).

There has been substantial interest in determining the relationships among inbreeding coefficients (Keller and Waller 2002) and levels of molecular genetic diversity (heterozygosity-fitness correlations, or HFCs; Coltman and Slate 2003) to fitness. Molecular metrics are commonly used as a surrogate for pedigree inbreeding coefficients (*f*), because the latter can be logistically difficult to obtain within natural populations. The most frequently used molecular metrics of heterozygosity in HFCs analyses include standardized multilocus heterozygosity (stMLH; Coltman et al. 1999), internal relatedness (IR; Amos et al. 2001) and standardized mean d^2 (Coulson et al. 1998). However, it has been suggested that the correlation between molecular heterozygosity and pedigree inbreeding coefficients is too weak to be of biological significance (Coltman and Slate 2003; Balloux et al. 2004; Slate et al. 2004; Hansson and Westerberg 2008). For wild canids, results vary even between studies of the same species. For example, Hedrick et al. (2001) reported a significant negative correlation between heterozygosity and inbreeding coefficients for the Scandinavian gray wolf (*Canis lupus*). However, Bensch et al. (2006) found no significant relationship between these metrics in the same species, which the authors attributed to natural selection for heterozygosity in this particular population.

The presence of significant, albeit modest HFCs has been reported in numerous studies, while a few investigations have found no relationships (Coltman and Slate 2003). Molecular metric assessments have led to HFC discoveries involving fitness traits such as fecundity (Amos et al. 2001), immunocompetence (Coltman et al. 1999), recruitment (Jensen et al. 2007) and survival (DaSilva et al. 2006). Although small populations of endangered species are most vulnerable to inbreeding and fitness reduction (Fitzpatrick and Evans 2009), most HFC studies have focused on large populations of common species (see Grueber et al. 2008 for review). Common species offer benefits in terms of large sample sizes and well understood natural histories. However, findings from such studies may not have much relevance to rare wildlife species that have experienced bottlenecks and, therefore, are highly susceptible to genetic diversity loss. HFCs are more likely to be detected in populations of such species because these populations are prone to: (1) an increased mean and variance of inbreeding coefficients due to prevalence of incestuous matings (Grueber et al. 2008); (2) more environmental stress that can intensify inbreeding depression, thereby increasing variance in fitness responses (DaSilva et al. 2006); and (3) a greater degree of linkage disequilibrium due to bottlenecks, selection or population admixture.

In this study, we tested the hypothesis that inbreeding coefficients and molecular heterozygosity predict fitness in a free-ranging, endangered species, the African wild dog (*Lycaon pictus*) in KwaZulu-Natal (KZN) province, South Africa. Approximately 5,700 wild dogs remain in Africa, living in a small fraction of their former range, with most populations isolated due to human encroachment, habitat loss and fragmentation (Woodroffe et al. 1997). In South Africa, nearly half of the estimated 400 wild dogs are found in small, fenced, protected areas managed as a metapopulation (Davies-Mostert et al. 2009). These reserves are scattered throughout the country and have limited carrying capacities, usually accommodating only one or two packs each (Mills et al. 1998). Fencing generally is sufficiently 'porous' to allow single-sex groups to disperse from the natal pack and travel as far as hundreds of kilometers. However, the sparsity of protected areas and the human-dominated landscape prevent most dispersers from reaching a suitable, alternative reserve in other parts of the country (Woodroffe et al. 1997). Therefore, for the metapopulation conservation plan to be effective, wild dogs often are physically translocated between areas to mimic natural dispersal with the objective to enhance overall species demography while sustaining genetic diversity (Mills et al. 1998).

Interestingly, this coordinated and intensive management plan is conducted without information on the genetic status or viability of the African wild dog metapopulation. There is a general assumption that some inbreeding has occurred in selected packs, although at unknown levels, and that wild dogs with reduced genetic diversity are 'less fit' than more heterozygous counterparts. The expression of deleterious effects has been demonstrated in inbreeding studies of wild canids (Liberg et al. 2005; Bensch et al. 2006; Hedrick and Fredrickson 2008), but these results are based on small sample sizes and low statistical power (Frankham 2009). The evidence is more definitive from studies of captive canids where inbreeding has been associated with increased proportions of malformed spermatozoa (Mexican wolf, *C. l. baileyi*; Asa et al. 2007), reduced fecundity (Fredrickson et al. 2007), blindness and reductions in juvenile weight and longevity (Scandinavian wolf; Laikre and Ryman 1991). Inbreeding has been linked to reduced immunocompetence in some non-canid species (O'Brien and Evermann 1988; Acevedo-Whitehouse et al. 2003). If inbreeding affects disease susceptibility in African wild dogs as well, this could markedly impact species persistence because rabies, canine distemper and parvo- viruses already have caused significant mortalities in multiple populations (Gascoyne et al. 1993; Alexander and Appel 1994; Cleaveland et al. 2000). Decreases

in immunocompetence may leave wild dogs vulnerable to disease-related mortalities as a result of reduced diversity in genes of the major histocompatibility complex (MHC) (Marsden et al. 2009).

In this study, we used detailed information generated from our long-term involvement in establishing and monitoring a reintroduced population of wild dogs in South Africa. We worked with conservation authorities to translocate animals and then monitored 257 individuals representing 10 distinctive packs. Although translocations and dispersal events resulting in natural pack formations have increased the KZN population since 2001, occasional observations of interbreeding between close relatives have been made in the field. To explore if these events impacted genetic diversity or fitness traits, we analyzed the relationships between inbreeding coefficients and molecular metrics and then determined if either metric was predictive of fitness. For the purpose of this study, we considered fitness of wild dogs to be largely reliant on the ability of an individual to find a breeding pack (McNutt 1996), be dominant (Creel and Creel 2002), produce offspring and survive (Buettner et al. 2007). We included inclusive reproductive success in our analyses because wild dogs are mostly obligate cooperative breeders (Courchamp et al. 1999), and we sought a precise measure of lifetime reproduction through each individual's contribution via actual breeding and/or helping to raise related kin. Finally, regardless of outcome on the relatedness issues described above, we predicted that this study of a major population of an endangered species would be important because it (1) begins a substantial database useful for long-term genetic and fitness monitoring of a reintroduced wild carnivore and (2) provides objective data informative to conservation managers on minimal best practices to sustain genetic diversity and future population health.

Materials and methods

Study population

Intensive demographic and behavioral monitoring was conducted for the reintroduced wild dog population in KZN province, South Africa from January 2001 through October 2008. The population was established in the 1980s with initial reintroductions to Hluhluwe-iMfolozi Park (HiP). Additional translocations were made to HiP in 1997 (Somers and Maddock 1999), 2001 and 2003 (Gusset et al. 2006), as well as to two other semi-connected protected areas in KZN province in 2005 and 2006 (Davies-Mostert et al. 2009). Most of these approximately 20 founders were wild-caught dogs captured on private farmlands after conflict with livestock and game farmers, while two individuals were sourced from Kruger National Park, and still others were captive-bred dogs that were bonded with wild-caught dogs to improve the hunting skills of reintroduced packs (Gusset et al. 2006). It was highly unlikely that source populations were comprised of significant numbers of inbred individuals as it is well established that this species displays several inbreeding avoidance tactics, including long-distance dispersal (McNutt 1996; Girman et al. 1997). In addition, when pedigree information was known, only sources of wild dogs without a probable history of inbreeding were selected as founders (Mills et al. 1998). Each founder pack was comprised of males derived from a different locality than the females in the pack, and founders of newer packs were not chosen from areas where previous founders were sourced.

Over the 7 year study period, the KZN wild dog population grew steadily through reintroductions and natural pack formations and from one protected area with two breeding packs comprising seven individuals to three protected areas with nine breeding packs and 88 dogs (Somers et al. 2008). In all, a total of 257 individuals were identified and present in the KZN study population over our study interval. One to four individuals per pack were fitted with VHF radio-collars so that groups (including dispersers) could be located by radio-telemetry. Data on pack composition (number of animals, age and gender structure), life histories (births, dispersals, pack formations, deaths), dominance (the hierarchy of each sex in a pack) and breeding status (mating, denning) were collected at least once and as often as 10 times monthly. Individual wild dogs were recognized by unique coat patterns and photographs to facilitate identifications. Pups were first counted, identified and sexed upon emergence from the den at 3 month of age. Within each pack, the alpha male and female were determined based on: (1) reciprocal male and female scent marking behaviors; (2) obvious coincidental male and female movements; and (3) dominance and mutual offense and defense in agonistic encounters with other adult pack members (Girman et al. 1997). Dominance tenure was measured as the proportion of the time spent as an alpha-ranked individual in a breeding pack and was categorized as 'always dominant', 'dominant at sometime', or 'never dominant'. The lifespan of each individual in the population was usually known with precision to within 1 month of birth and death. When animals disappeared from protected areas, it often was possible to differentiate between dispersal and mortality events because dispersers were intermittently sighted and reported. Additionally, it was extremely unlikely that unseen, dispersing individuals would survive for long periods of time outside of protected areas due to a lack of prey and potential mates. It was assumed that any dominant female not observed with her

pack for two consecutive sightings had died because alpha females do not disperse (Creel and Creel 2002).

Genetic sampling and genotyping

Sampling for genetic analyses was conducted from January 2003 through January 2008 using a combination of hands-on and non-invasive approaches. Specific sample collection, immobilization techniques and DNA extraction methods are described in Spiering et al. (2009, 2010). Briefly, tissue and blood samples were obtained opportunistically during immobilization procedures for translocation and collaring, or when the occasional wild dog carcass was discovered. A larger and more representative sample was obtained by using non-invasive fecal DNA assessments (n = 113 individuals from 10 packs). Fecal DNA was extracted from scat with the QIAamp DNA Stool Mini Kit and from tissue and blood with the QIAamp Tissue and Blood Kit (QIAGEN). Genotyping was conducted using 19 microsatellite loci selected from the 2006 ISAG (International Society for Animal Genetics) domestic dog (*Canis familiaris*) panel that were consistent with those used in other wild dog genetic studies in southern Africa. All individuals were typed at 17 dinucleotide microsatellite loci and two tetranucleotide loci (Table 1). These loci are commonly used for determining parentage in domestic dogs and, therefore, were known to be highly polymorphic and distributed throughout the genome. Polymerase chain reaction (PCR) protocols and the elimination of genotyping and sampling errors in our fecal DNA analysis are addressed in detail in Spiering et al. (2009). In all, 87.6% of individuals (n = 99) were genotyped at all 19 loci, 8.9% at 17 or 18 loci and the remaining four individuals at 13 or more loci.

Parentage analysis

Parentage of offspring was assumed from pack composition only in cases where a single pair of adults was present and behavioral observations confirmed breeding status. All other parentage assignments were made based on a combination of longitudinal behavioral observations plus molecular genetic data. Tests for deviation from Hardy–Weinberg equilibrium and parentage analyses were completed using the likelihood-based approach in the program CERVUS (version 3.0) and are described in detail in Spiering et al. (2010). Locus INU030 was excluded from the parentage analyses because a significantly lower than expected frequency of heterozygotes was detected, indicating a high incidence of null alleles. No other locus deviated from Hardy–Weinberg equilibrium. The simulation program in CERVUS was used to establish the critical difference in LOD scores (natural logarithm of the likelihood ratio) between the first and second most likely candidate parents at >95% confidence. For the 18 loci used in the analysis, the overall probability of exclusion was 0.991 for the first parent and 0.999 for the second.

Table 1 Microsatellite loci used for estimating molecular heterozygosity metrics and determining parentage in free-ranging African wild dogs

Locus	Chromosome	Allele sizes	Number of alleles	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)
AHT137	CFA11	131–147	5	0.81	0.71
AHTH130	CFA36	117–125	6	0.46	0.47
AHTH171	CFA06	217–225	6	0.80	0.72
AHTH260	CFA16	246–254	5	0.52	0.61
AHTK211	CFA26	89–91	2	0.61	0.49
AHTK253	CFA23	298–306	6	0.77	0.73
CXX279	CFA22	116–118	3	0.26	0.23
FH2054	CFA12	128–140	4	0.80	0.66
FH2328	CFA29	194–220	10	0.76	0.81
FH2848	CFA02	232–240	5	0.72	0.71
INRA21	CFA21	97–101	3	0.49	0.41
INU030	**CFA12**	**144–150**	**6**	**0.55**	**0.60**
INU055	CFA10	208–216	4	0.76	0.67
LEI004	CFA37	95–99	3	0.58	0.61
REN54P11	CFA18	234–246	5	0.71	0.67
REN105L03	CFA11	235–245	7	0.81	0.80
REN162C04	CFA07	194–200	6	0.74	0.73
REN169D01	CFA14	208–212	3	0.29	0.28
REN247M23	CFA15	254–256	2	0.27	0.27

Bold font indicates a locus not in Hardy–Weinberg equilibrium and, therefore, excluded from parentage analyses

Inclusive fitness

Total lifetime reproductive success or inclusive fitness was based on the number of offspring parented by each animal (direct fitness) plus the number of offspring belonging to closely-related pack members that the individual assisted in raising (indirect fitness). For this calculation, we used relatedness values of 0.50 for offspring and full siblings, 0.25 for half-siblings, nieces and nephews and 0.12 for grand-offspring, then multiplied these values by 2 to convert to units of offspring equivalents (Grafen 1982). All pairwise genetic relatedness values were estimated using the program KINSHIP (version 1.3.1).

Inbreeding coefficients

The inbreeding coefficient (f) of each wild dog was calculated from the pedigree using FSPEED (version 2.04; Tenset Technologies Limited). Because limited pedigree information may lead to underestimations of f (Keller 1998), individuals were assigned inbreeding coefficient values only in cases where grandparents were confirmed using parentage analyses or when behavioral and demographic records allowed confidence in pedigree data (n = 181 wild dogs, 10 packs). Given the origin and history of reintroduction of this population, inbreeding levels were likely approximately $f = 0$ before the last two generations. Because 257 wild dogs were present during our monitoring interval, we derived inbreeding coefficients for 70.4% of the collective population.

Molecular metrics of heterozygosity

Heterozygosity was calculated for all genotyped individuals (n = 113) for three different molecular measures of genome-wide diversity. Of the 181 wild dogs assigned f values, 85 (from eight packs) also were genotyped to include corresponding molecular metrics. Standardized multilocus heterozygosity (stMLH) was calculated based on the proportion of loci genotyped for a given individual that was heterozygous divided by mean heterozygosity in the population at the same loci (Coltman et al. 1999; Slate et al. 2004). IR reflects the relatedness of the parents of an individual by determining the degree of allele-sharing relative to random expectations across all loci (Amos et al. 2001). Standardized mean d^2 (hereafter referred to as d^2) is a measure focused on events deeper in the pedigree than individual heterozygosity (Hedrick et al. 2001) and is based on the genetic distance between parental gamete genomes (Coulson et al. 1998, 1999). d^2 was calculated for each individual as the squared distance in repeat units between two alleles at a given locus, averaged over all loci typed for that individual (Coulson et al. 1999) and then standardized by dividing each value by the maximum observed at that locus (Hedrick et al. 2001). This metric is used with the assumption that microsatellites evolve under the stepwise mutation model (SMM; Valdes et al. 1993). Therefore, departure from SMM was tested for all loci with the program BOTTLENECK (Cornuet and Luikart 1996). The stMLH, IR and d^2 values were calculated using an EXCEL macro written by Amos et al. (2001).

Statistical analyses

The relationship between pedigree inbreeding coefficients and microsatellite-based metrics were compared by linear regression. To test the strength of the correlation between f and stMLH, we compared our observed linear correlation to the predicted relationship using the model of Slate et al. (2004) based on the mean population f as well as the variance in f and heterozygosity. We used generalized linear mixed models (GLMMs) to evaluate the relationship between molecular metrics (stMLH, IR and d^2) or inbreeding coefficients (f) on: (i) breeding pack membership, (ii) tenure of pack dominance, (iii) individual inclusive fitness and (iv) lifespan. High correlations between the molecular metrics (Table 2) precluded joint inclusion into a single model. Molecular metrics and f were not considered within a single joint model due to too few individuals with available data for both measures and fitness traits.

All models were fit using Laplace maximum likelihood approximation and all included pack identity as a random effect (Bolker et al. 2008). GLMMs were used to evaluate breeding pack membership (binomial, logit), inclusive fitness (poisson, log-link) and lifespan (poisson, log-link) in relation to the fixed effect predictors of sex, dominance and one molecular metric (stMLH, IR or d^2) or f. All models included pack identity as a random effect. Pack dominance was treated as a multinomial response variable in generalized linear models incorporating sex, one molecular metric (stMLH, IR or d^2) or f, and pack as fixed effect predictors. For lifespan analyses, inbreeding coefficients were coded as two categorical predictors of inbred ($f \geq 0.25$) or outbred ($f = 0$), because our dataset primarily consisted of individuals with $f = 0.25$ or $f = 0$.

Table 2 Linear correlation coefficients (r) between inbreeding coefficients and molecular metrics of heterozygosity within African wild dog individuals (n = 85)

	stMLH	d^2	IR
f	−0.37**	−0.18	0.35*
stMLH	–	0.75**	−0.99**
d^2	–	–	−0.76**

An asterisk indicates a correlation that is significantly different from zero with * $P < 0.05$ and ** $P < 0.001$

Breeding pack membership and dominance models included dead animals only, as both of these life history events may occur at any time in the life of a wild dog. Animals that died before joining a breeding pack were excluded from models for dominance as wild dogs rarely achieve dominance in their natal packs (Spiering et al. unpublished data). For models of inclusive fitness, only individuals that were dead and had survived to at least 12 month (yearlings) were included in analyses, as this allowed calculating lifetime reproductive success of mature individuals. In the lifespan analyses, only dead individuals that had survived beyond 12 month and for which sex was known were included. Living individuals were excluded from the analysis without introducing bias since the relatively short average lifespans of wild dogs in our population (2 year) and others (Creel and Creel 2002) meant that the majority of living animals were young (63.3% of the 98 dogs were <2 year old). Additionally, there was no statistical difference between the age of living individuals at the end of the study (mean = 1.91 ± 1.78 year) and the lifespans of dead counterparts (2.00 ± 1.94 year; Wilcoxon test, $T_{254} = -0.24$, $P = 0.71$). The live and dead groups also did not differ in inbreeding coefficients (0.03 ± 0.06 and 0.09 ± 0.12, respectively; $T_{214} = -4.54$, $P = 0.14$) or stMLH (1.06 ± 0.18 and 1.02 ± 0.20, respectively; $T_{105} = 0.88$, $P = 0.57$).

Model selection

We used Akaike's Information Criterion (AIC) to evaluate among candidate models based on all combinations of predictor variables in addition to an 'intercept-only' approach. All combinations represented reasonable a priori hypotheses and were required to calculate factor weights. Model selection was based on AIC for small samples (AIC_c), where we considered models with $\Delta AIC_c \leq 2$ to be well supported by the data (Burnham and Anderson 2002). All statistical analyses were conducted using the Program R (version 2.8.1) and JMP software version 3.2.2 (SAS Institute Incorporated), with means given ± standard deviation of the mean.

Results

Pedigrees and parentage assignments confirmed via genetic analyses allowed the estimation of inbreeding coefficients for 181 African wild dogs using a minimum of two generations of data. The mean and variance in *f* for this population was 0.074 and 0.008, respectively, with 37.5% of wild dogs with known pedigrees having a non-zero inbreeding coefficient. Values of *f* ranged from 0 (n = 115 animals) to 0.281 (n = 4; Fig. 1a).

A total of 113 wild dogs were genotyped using the panel of polymorphic microsatellite loci to quantify individual heterozygosity using stMLH, IR and d^2. Tests of mutation models using BOTTLENECK revealed that the distribution of the microsatellites did not depart from the expected distribution under the stepwise mutation model ($P = 0.73$, n = 113). Within the study population, the mean stMLH was 1.01 ± 0.18, the mean IR was 0.39 ± 0.10, and d^2

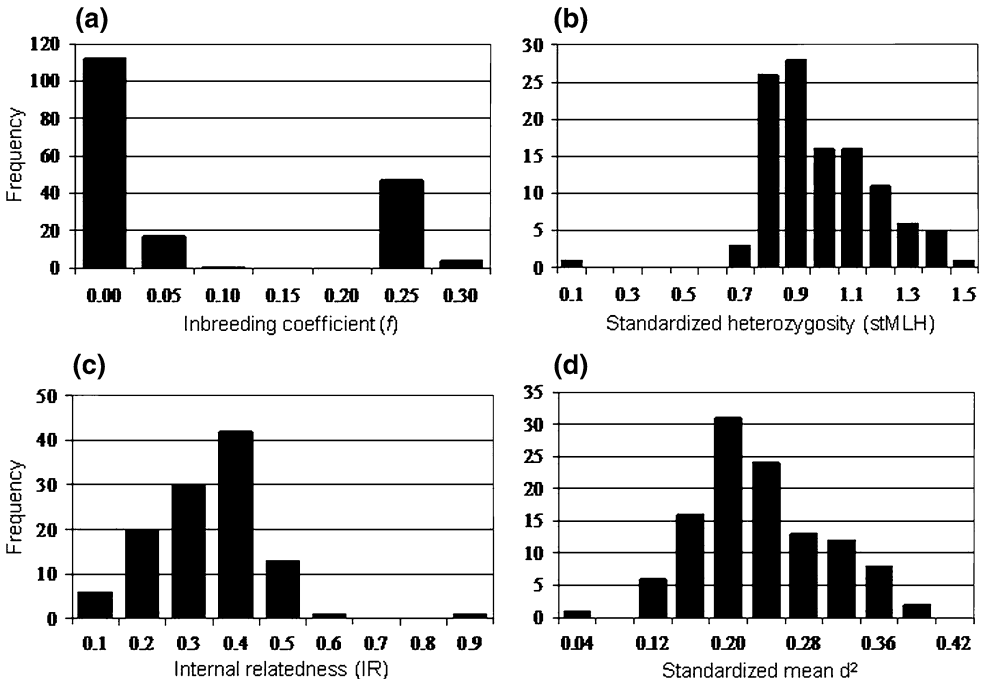

Fig. 1 Frequency distributions of **a** inbreeding coefficients (*f*; n = 181 African wild dogs); **b** standardized multilocus heterozygosity (stMLH); **c** internal relatedness (IR); and **d** standardized mean d^2 (d^2; n = 113 African wild dogs)

was 0.21 ± 0.06 with distributions of the molecular metrics approximately normal (Fig. 1b–d).

In terms of life history metrics, we observed that 25.3% (n = 65) of wild dogs in this population successfully joined or formed a breeding pack, and 13.3% (n = 34) of all individuals secured the alpha position in the dominance hierarchy during their lifetime. Mean offspring number per wild dog was 1.8 ± 7.0 pups with inclusive reproductive success of individuals averaging 9.6 ± 6.8 total young. Average lifespan for all dogs was 2.0 ± 1.9 year, with individuals that attained dominance status in breeding packs living longer (4.7 ± 2.2 year; Wilcoxon test, $T_{176} = -6.0$, $P < 0.0001$).

Relationship between inbreeding coefficient and heterozygosity

The correlation between f and d^2 was not statistically significant, though the remaining correlations were ($P < 0.05$), with stMLH and IR most strongly related ($r = -0.99$; Table 2). Linear correlations also revealed that stMLH was negatively correlated to f ($r = -0.37$, $P < 0.001$; Fig. 2), with the relationship similar to but slightly weaker than predicted under the model by Slate et al. (2004) ($r_{predicted} = -0.44$).

As multilocus heterozygosity can only reflect genome-wide diversity (i.e. general effects of inbreeding) if individual loci are correlated (Balloux et al. 2004), we further explored whether stMLH was correlated among loci in our panel. Upon estimating heterozygosity of 10 random subsets of loci, results revealed only a modest, non-significant correlation between genetic markers ($r = 0.16$, $r^2 = 2.4\%$). Thus, it appeared that heterozygosity was an inadequate predictor of inbreeding coefficients in this African wild dog population.

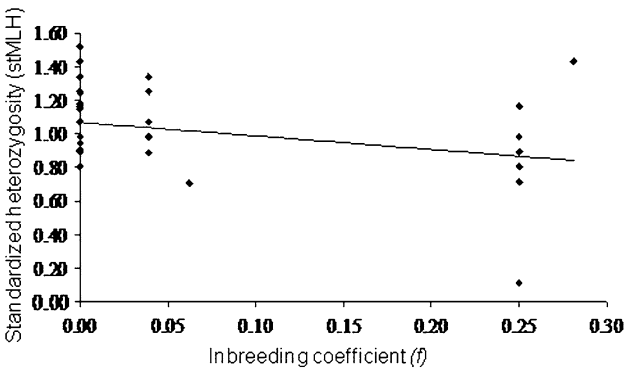

Fig. 2 The relationship between standardized multilocus heterozygosity (stMLH) measured using 19 microsatellites and inbreeding coefficient (f) of 85 KZN African wild dogs

Influence of molecular metrics and f on fitness traits

The GLMM analyses to evaluate the relationship between the three molecular metrics and fitness measures yielded the same models both in rank and magnitude of support. Therefore, to avoid redundancy, we present here only the stMLH results.

Breeding pack membership

The most informative model of the GLMM analyses of molecular metrics and breeding pack membership was the intercept only model (Table 3a). In analyses that only included f-coefficients, the most supported model included sex and pack (Table 3e), with males having a

Table 3 Summary of most informative models from molecular metric analyses (a–d) and f-coefficient analyses (e–h)

Models	AIC_c	ΔAIC_c	K
Molecular metric analyses			
(a) Breeding pack membership			
Intercept-only	167.9	0.0	6
Sex	78.4	1.49	2
(b) Tenure of dominance			
Sex	126.6	0.0	4
Intercept-only	127.3	0.8	2
stMLH	128.1	1.5	6
(c) Inclusive fitness			
Dominance + pack	172.0	0.0	4
Dominance	173.8	1.8	3
Sex + dominance + pack	173.9	1.9	5
(d) Lifespan			
Dominance + pack	83.3	0.0	4
Sex + dominance + pack	85.0	1.1	5
stMLH + dominance + pack	85.8	1.9	5
f-coefficient analyses			
(e) Breeding pack membership			
Sex + pack	99.5	0.0	4
(f) Tenure of dominance			
Sex	96.2	0.0	4
(g) Inclusive fitness			
Dominance + pack	204.9	0.0	4
Sex + dominance + pack	205.0	0.1	5
Sex + pack	205.2	0.3	4
Pack	205.5	0.6	3
f + dominance + pack	206.7	1.8	6
(h) Lifespan			
f + dominance + pack	92.9	0.0	5

AIC_c is the AIC value corrected for small sample sizes; ΔAIC_c is the change in AIC_c between that model and the best fitting model; and K is the number of parameters associated with that model. All models with a ΔAIC_c less than 2.0 are shown

lower probability of joining a breeding pack than females ($\hat{\beta} = -0.08$, 95% CI: −0.62, 0.46).

Tenure of pack dominance

For both the molecular metric and *f* GLMM analyses, the top model predicting duration of pack dominance was the single predictor model that included sex (Table 3b, f), where females tended to have longer durations of dominance than males ($\hat{\beta} = 0.44$, 95% CI: 0.43, 1.33), as previously reported (Spiering et al. 2010). When only females were considered, neither *f* nor pack identity was predictive of the duration of dominance.

Inclusive fitness

The most informative model resulting from our GLMM analyses of molecular metrics describing inclusive fitness included dominance and pack (Table 3c). Dominance increased inclusive fitness ($\hat{\beta} = 0.80$, 95% CI: 0.55, 1.05), and pack effects accounted for 14% of the total variance. Similarly, in *f*-coefficient only analyses, the top model included dominance and pack (Table 3g), with dominance increasing inclusive fitness ($\hat{\beta} = 0.51$, 95% CI: 0.22, 0.80) and pack effects accounting for 29% of the variance. However, models that included sex had similar support, where males had lower inclusive fitness than females ($\hat{\beta} = -0.35$, 95% CI: 0.22, 0.80).

Lifespan

When lifespan was examined in relation to molecular metrics, the most supported model included dominance (Table 3d), where dominant individuals had longer lifespans than subordinates ($\hat{\beta} = 0.41$, 95% CI: 0.29, 0.53). Pack effects accounted for 5.8% of the total variance in lifespan. For the lifespan analyses in relation to *f*, the most supported model incorporated dominance and *f* (Table 3h). Individuals that became dominant for at least part of their time in a breeding pack had longer lifespans than subordinates ($\hat{\beta} = 0.34$, 95% CI: 0.2, 0.48). Outbred wild dogs with $f = 0$ also lived longer than inbred counterparts ($\hat{\beta} = 0.61$, 95% CI: 0.47, 0.75) with the latter compromised in longevity by 0.83 years on average. Pack effects accounted for 6.8% of the total variance in lifespan.

Discussion

Here we have presented one of the few studies of an endangered species that has integrated data on inbreeding coefficients from pedigrees, molecular metrics of heterozygosity and multiple fitness traits. The remaining small, segregated populations of African wild dogs, like the reintroduced population we are studying in South Africa, are vulnerable to matings between close relatives that can lead to loss of genetic diversity and inbreeding depression. Because most of the founders in our study were released after 1997, and because average generation time is 4 year (O'Grady et al. 2008), so far it has been possible to evaluate only a few generations. Nonetheless, our genetic analyses confirmed that inbreeding indeed has occurred, but only in a limited number of packs within the KZN population. Perhaps for this reason or because our study has limited statistical power due to the ever-present challenge of securing large sample sizes for endangered species, inbreeding depression was not detected in the fitness traits of breeding pack membership, tenure of dominance or inclusive fitness. Even so, results suggested that inbreeding already may have reduced longevity of inbred wild dogs by as much as 10 month. However, as many individuals with an $f \geq 0.25$ originated from a single pack (called iMfolozi), pack-specific effects and inbreeding were confounded. Much of the inbreeding in the KZN population has been associated with the iMfolozi pack as a mother–son pair produced large litters of offspring in four consecutive years. Although our interpretation of the fitness effects due to inbreeding were mostly confined to this subset of the population with reduced heterozygosity, it nonetheless clearly demonstrated how rapidly inbreeding depression can affect fitness.

Our analyses indicated that non-genetic or non-inbreeding related traits, including dominance, sex and pack, were also important predictors of fitness metrics in this population. Dominance was included in the top model for inclusive fitness, which was logical given that dominant individuals parent a majority of offspring (Girman et al. 1997; Spiering et al. 2010) and tend to live longer (Spiering et al. 2010). Dominance also predicted lifespan in our analyses, an expected trend, as the chance of becoming an alpha individual is known to increase with age in this and other wild dog populations (Creel and Creel 2002; Spiering et al. 2010). Results from earlier studies may explain why male wild dogs are less likely to establish breeding packs than females. For example, it is known that: (1) males disperse farther distances than females (McNutt 1996) and may die before finding mates as survival success decreases with distance from the source population (Lindsey et al. 2004); and (2) male bias in adult age classes creates a situation where male disperser groups are less successful in finding similar female groups due to their scarcity (Spiering et al. 2010). In our present evaluation, pack association influenced inclusive fitness, with the highest values belonging to individuals within two packs. This was

not likely due to habitat quality differences because inbred and outbred packs overlapped in portions of their home ranges and were not temporally separated. Rather, we suspect that tenure played an important role, with the bigger, longer-established packs comprised of individuals with the highest inclusive fitness, including significant experience in raising multiple large litters of related pups. In contrast, more recently formed packs were small in size and more likely to produce litters with fewer pups (McNutt and Silk 2008), thereby limiting the indirect fitness of pack members.

Because molecular evidence of heterozygosity was correlated with inbreeding coefficients, we expected these metrics to be predictive of variation in fitness traits, especially lifespan. Standardized multilocus heterozygosity in particular was negatively correlated to f values, and the relationship was similar to that predicted by Slate et al. (2004). It is well established that the strength of the correlation between genome-wide heterozygosity and marker-based heterozygosity is influenced by the amount of historic inbreeding in a population (Aparicio et al. 2007; Grueber et al. 2008; Szulkin et al. 2010). Because small populations of endangered species such as the African wild dog have reduced effective population sizes, inbreeding is often common. Therefore, marker-based heterozygosity should more accurately reflect genome-wide heterozygosity and, thus, more precisely indicate inbreeding levels in these populations (Fitzpatrick and Evans 2009). This, combined with a greater degree of linkage disequilibrium (due to bottlenecks, selection or population admixture) and the likelihood of increased environmental stress for threatened species that can intensify inbreeding depression (Grueber et al. 2008), gives substantial support for the possible detection of significant HFCs in this species. Although we observed breeding between close kin and inbreeding coefficients were negatively correlated with fitness, none of the three molecular metrics of genetic diversity were predictive of fitness traits.

The absence of HFCs suggested that the small effective population size and the observed, occasional incestuous matings had not yet occurred at sufficient frequencies to create a signature of reduced genome-wide heterozygosity detectable by our markers and sample size. The weak correlation among subsets of loci also suggested that heterozygosity averaged across markers (stMLH) did not indicate major loss of diversity due to inbreeding (Balloux et al. 2004). Additionally, the lack of significant HFCs suggested that any linkage among our microsatellites and quantitative loci did not enhance these relationships caused by inbreeding, at least sufficiently so to be clearly detected (Szulkin et al. 2010). Lastly, although the number of individuals included in our study comprised a significant proportion (circa 65%) of the entire wild dog population of South Africa, the sample size possible with these endangered animals may not provide sufficient power to detect these relationships (Coltman and Slate 2003). This was demonstrated by the wide confidence intervals observed in our results. This issue is common in most studies where (1) inbreeding is not evenly distributed throughout the pedigree, (2) few founders initiated the population and (3) big sample sizes are impossible to secure due to limited numbers of extant or captive animals (Kalinowski and Hedrick 1999).

Our study demonstrated that even in a small population of an endangered species, the conditions under which statistically significant HFCs are detectable make it difficult to assume that molecular metrics are an accurate reflection of real inbreeding levels. This issue appears especially relevant as molecular metrics are commonly used as a surrogate for inbreeding coefficients because pedigree information is often unavailable and difficult to reconstruct via molecular parentage analyses (Coltman and Slate 2003). The results of our molecular metrics analyses were derived from a panel of 19 microsatellite loci, which is larger than those used in many other studies of endangered or wild species (e.g., Coulson et al. 1999). Nevertheless, our findings were consistent with recent studies suggesting that the correlation between marker-based heterozygosity and inbreeding levels may often be too weak to be of biological significance (Coltman and Slate 2003; Hansson and Westerberg 2008). However, we are not prepared to take this latter position because, to-date, most of our pedigrees were only two generations deep. Balloux et al. (2004) suggested that this level can be sufficient for making broad-scale inferences because recent events have a disproportionately large influence on inbreeding coefficients. However, in such cases, f values may have been underestimated if founders were derived from populations that had already accumulated substantial inbreeding or had been subject to strong genetic drift. In this case, it is unlikely that source populations included high numbers of inbred wild dogs since previous studies have shown that inbreeding has been minimal (McNutt 1996; Girman et al. 1997) and, in general, the genetic diversity of wild dogs has been retained despite recent habitat loss and population declines (Girman et al. 2001). Furthermore, founders were carefully selected to avoid known inbred individuals and were sourced from different areas to avoid including related individuals. Including five generations or more in future studies, as recommended by Balloux et al. (2004), will provide invaluable data to more accurately estimate inbreeding coefficients, which should better correlate with molecular metrics. While neutral molecular markers may provide a means of rapid assessment of diversity, inbreeding coefficients remain most predictive of fitness. Therefore, pedigree information should be continually

recorded for endangered populations, as inbreeding may have severe impacts on the fitness of these already struggling species (Frankham et al. 2002).

Concerns regarding the vulnerability of endangered canids to reduced genetic diversity are warranted. A particularly poignant example is that of an extirpated Scandinavian population of wild wolves that was repatriated with natural immigration and began with only three founders (Liberg et al. 2005). Although now having increased to approximately 150 individuals (Bensch et al. 2006), reduced pup survival (Liberg et al. 2005) and a higher incidence of physical deformities (Raikkonen et al. 2006) suggest that inbreeding depression is influencing the growth of this population. However, Bensch et al. (2006) found that loss of genetic diversity was slowed because breeding establishment was positively correlated with heterozygosity. Nonetheless, inbreeding is known to exert a significant influence in wild canids, especially those maintained ex situ, where genetic management protocols are lacking or fail to be appropriately implemented. For example, several generations of inbreeding within a captive Mexican wolf population led to certain males producing abnormally high proportions of severely deformed spermatozoa with poor motility (Asa et al. 2007). Deformed spermatozoa impede reproductive function and are commonly associated with reduced genetic variation and inbreeding (Fitzpatrick and Evans 2009). However, there is no evidence that these types of physiological anomalies are occurring yet within the KZN population. Even males with an inbred lineage have produced multiple, normal-sized litters (up to 14 pups each).

Conservation implications

In the past, little attention has been directed toward recording accurate pedigrees or monitoring genetic status in the South African wild dog metapopulation, despite the heavy influence of genetic characteristics on management decisions. Wild dogs that were presumed to be inbred have been isolated and not translocated to new areas to reproduce because they were presumed to be less fit, despite the lack of empirical evidence. Our results suggested that removing these individuals may be premature, as these 'inbred' animals may have demographic value in the metapopulation. Although we have observed slightly reduced longevity, to date there has been no evidence that these wild dogs are any less capable of finding breeding packs, becoming dominant or producing healthy offspring. With so few wild dogs remaining in South Africa (and throughout Africa), avoiding inclusion of any individual may be only defensible if the data clearly indicated that there was a potential viability risk to the population (or species). However, we recommend caution when making final decisions on the conservation utility of 'inbred' wild dogs, as this will require more longitudinal data, in part, because increasing genetic diversity in populations is challenging and requires extensive management. Within each population, we strongly recommend that emphasis be placed on recording detailed pedigrees and conducting genetic analyses to provide objective measures of heterozygosity and parentage. Knowledge of inbreeding levels will be an important predictor of management success and, more importantly, will provide guidance for future translocations, releases or other intercessions. In this context, it only is logical to recommend that studies on small and fragmented populations of endangered species include deeper pedigrees, genetic status, life histories and physiological metrics whenever possible. Integrating the latest tools and information gleaned from conservation genetics can only improve our ability to better understand the complex phenomenon of inbreeding depression and its importance in making critical management decisions.

Acknowledgments We thank Ezemvelo KwaZulu-Natal Wildlife, especially the management teams at Hluhluwe-iMfolozi Park and the uMkhuze section of iSimangaliso Wetland Park. We also appreciate the assistance of Thanda Private Game Reserve and the wildlife management team. We are grateful to Rob Fleischer, Emily Latch, Sarah Haas, Kalon Armstrong and Nancy Rotzel of the Smithsonian's Conservation Biology Institute for help with laboratory protocols and procedures. Sarah Arnoff, Jan Graf, Gabriella Flacke, Mariana Venter, Carla Naude-Graaff, Sboniso (Zama) Zwane, Brendan Whittington-Jones, Chris Kelly and Warren Becker provided invaluable assistance in the field. We thank Robert Sivinski and Scott Wilson of the Smithsonian's Migratory Bird Center for statistical advice. This research was supported by the Smithsonian Institution Undersecretary for Science Endowment Fund, the University of Pretoria, Rotterdam Zoo Thandiza Fund, Humboldt State University Sponsored Program Foundation, Conservation Endowment Fund of the Association of Zoos and Aquariums, Disney Wildlife Conservation Fund, Knowsley Safari Park, DST-NRF Centre of Excellence for Invasion Biology, Khaki Fever Work Wear, Pittsburgh Zoo Conservation Fund and the Morris Animal Foundation. International travel was generously provided by British Airways.

References

Acevedo-Whitehouse K, Gulland F, Greig D, Amos W (2003) Inbreeding: disease susceptibility in California sea lions. Nature 422:35

Alexander KA, Appel MJG (1994) African wild dogs (*Lycaon pictus*) endangered by a canine distemper virus epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. J Wildl Dis 30:481–485

Amos W, Wilmer JW, Fullard K et al (2001) The influence of parental relatedness on reproductive success. Proc R Soc Lond B 268: 2021–2027

Aparicio JM, Ortego J, Cordero PJ (2007) Can a simple algebraic analysis predict markers-genome heterozygosity correlations? J Hered 98:93–96

Asa C, Miller P, Agnew M et al (2007) Relationship of inbreeding with sperm quality and reproductive success in Mexican gray wolves. Anim Conserv 10:326–331

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? Mol Ecol 13:3021–3031

Bensch S, Andren H, Hansson B et al (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. PLoS One 1:e72

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JS (2008) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Buettner UK, Davies-Mostert HT, du Toit JT, Mills MGL (2007) Factors affecting juvenile survival in African wild dogs (*Lycaon pictus*) in Kruger National Park, South Africa. J Zool (Lond) 272:10–19

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretical approach, 2nd edn. Springer-Verlag, New York

Charpentier MJE, Williams CV, Drea CM (2008) Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence and survivorship. Conserv Genet 9:1605–1615

Cleaveland S, Appel MJG, Chalmers WSK, Chillingworth C, Kaare M, Dye C (2000) Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. Vet Microbiol 72:217–227

Coltman D, Slate J (2003) Microsatellite measures of inbreeding: a meta-analysis. Evolution 57:971–983

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53:1259–1267

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Coulson T, Pemberton JM, Albon S et al (1998) Microsatellites reveal heterosis in red deer. Proc R Soc Lond B 265:489–495

Coulson T, Albon S, Slate J, Pemberton JM (1999) Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. Evolution 53:1951–1960

Courchamp F, Grenfell B, Clutton-Brock T (1999) Population dynamics of obligate cooperators. Proc R Soc Lond B 266:557–563

Creel S, Creel NM (2002) The African wild dog: behavior, ecology and conservation. Princeton University Press, Princeton

DaSilva A, Luikart G, Yoccoz NG, Cohas A, Allaine D (2006) Genetic diversity-fitness correlation revealed by microsatellite analyses in European marmots (*Marmota marmota*). Conserv Genet 7:371–382

Davies-Mostert HT, Mills MGL, Macdonald DW (2009) A critical assessment of South Africa's managed metapopulation recovery strategy for African wild dogs. In: Somers MJ, Hayward MW (eds) Reintroduction of top-order predators. Wiley-Blackwell, London

Fitzpatrick JL, Evans JP (2009) Reduced heterozygosity impairs sperm quality in endangered mammals. Biol Lett 25:320–323

Frankham R (2009) Genetic considerations in reintroduction programmes for large terrestrial predators. In: Somers MJ, Hayward MW (eds) Reintroduction of top-order predators. Wiley-Blackwell, London

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Fredrickson RJ, Siminski P, Woolf M, Hedrick P (2007) Genetic rescue and inbreeding depression in Mexican wolves. Proc R Soc Lond B 274:2365–2371

Gascoyne SC, Laurenson MK, Lelo S, Borner M (1993) Rabies in African wild dogs (*Lycaon pictus*) in the Serengeti region, Tanzania. J Wildl Dis 29:396–402

Girman JG, Mills MGL, Geffen E, Wayne RK (1997) A molecular genetic analysis of social structure, dispersal and interpack relationships of the African wild dog (*Lycaon pictus*). Behav Ecol Sociobiol 40:187–198

Girman DJ, Vila C, Geffen E et al (2001) Patterns of population subdivisión, gene flow and genetic variability in the African wild dog (*Lycaon pictus*). Mol Ecol 10:1703–1723

Grafen A (1982) How not to measure inclusive fitness. Nature 298:425–426

Grueber CE, Wallis GP, Jamieson IG (2008) Heterozygosity-fitness correlations and their relevance to studies on inbreeding depression in threatened species. Mol Ecol 17:3978–3984

Gusset M, Slotow R, Somers MJ (2006) Divided we fail: the importance of social integration for the re-introduction of endangered African wild dogs (*Lycaon pictus*). J Zool (Lond) 270:502–511

Hansson B, Westerberg L (2008) Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects? Conserv Genet 9:73–83

Hedrick P, Fredrickson R (2008) Captive breeding and the reintroduction of Mexican and red wolves. Mol Ecol 17:344–350

Hedrick P, Fredricskon R, Ellegren H (2001) Evaluation of mean d^2, a microsatellite measure of inbreeding and outbreeding, in wolves with a known pedigree. Evolution 55:1256–1260

Jensen H, Bremset EM, Ringsby TH, Saether BE (2007) Multilocus heterozygosity and inbreeding depression in an insular house sparrow metapopulation. Mol Ecol 16:4066–4078

Kalinowski S, Hedrick PW (1999) Detecting inbreeding depression is difficult in captive endangered species. Anim Conserv 2: 131–136

Keller LF (1998) Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52: 240–250

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241

Laikre L, Ryman N (1991) Inbreeding depression in a captive wolf (*Canis lupus*) population. Conserv Biol 5:33–40

Liberg O, Andren H, Pedersen HC et al (2005) Severe inbreeding depression in a wild wolf population. Biol Lett 1:17–20

Lindsey P, du Toit JT, Mills MGL (2004) The distribution and population status of African wild dogs (*Lycaon pictus*) outside protected areas in South Africa. S Afr J Wildl Res 34:143–161

Marsden CD, Mable BK, Woodroffe R et al (2009) Highly endangered African wild dogs (*Lycaon pictus*) lack variation at the major histocompatibility complex. J Hered. doi: 10.1093/jhered/esp031

McNutt JW (1996) Sex-biased dispersal in African wild dogs, *Lycaon pictus*. Anim Behav 52:1067–1077

McNutt JW, Silk JB (2008) Pup production, sex ratios, and survivorship in African wild dogs, *Lycaon pictus*. Behav Ecol Sociobiol 62:1061–1067

Mills MGL, Ellis S, Woodroffe R et al (1998) Population and habitat viability assessment for the African wild dog (*Lycaon pictus*) in Southern Africa. Conservation Breeding Specialist Group. IUCN/Species Survival Commission, Apple Valley

O'Brien SJ, Evermann JF (1988) Interactive influence of infectious disease and genetic diversity in natural populations. Trends Ecol Evol 3:254–259

O'Grady J, Reed DH, Brook BW, Frankham R (2008) Extinction risk scales better to generations than to years. Anim Conserv 11: 442–451

Raikkonen J, Bignert A, Mortensen P, Fernholm B (2006) Congenital defects in a highly inbred wild wolf population (*Canis lupus*). Mamm Biol 71:65–73

Ralls K, Brugger K, Ballou J (1979) Inbreeding and juvenile mortality in small populations of ungulates. Science 206:1101–1103

Slate J, David P, Dodds KG et al (2004) Understanding the relationship between the inbreeding coefficient and multilocus

heterozygosity: theoretical expectations and empirical data. Heredity 93:255–265

Somers M, Maddock A (1999) Painted dogs of Zululand. Afr Wildl 53:24–26

Somers MJ, Graf JA, Szykman M, Slotow R, Gusset M (2008) Dynamics of a small re-introduced population of endangered wild dogs over 25 years: Allee effects and the implications of sociality for conservation. Oecologia 158:239–247

Spiering PA, Szykman Gunther M, Wildt DE, Somers MJ, Maldonado JE (2009) Sampling error in non-invasive genetic analyses of an endangered social carnivore. Conserv Genet 10:2005–2007

Spiering PA, Somers MJ, Maldonado JE, Wildt DE, Szykman Gunther M (2010) Reproductive sharing and proximate factors mediating cooperative breeding in the African wild dog (*Lycaon pictus*). Behav Ecol Sociobiol 64:583–592

Szulkin M, Bierne N, David P (2010) Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64:1202–1217

Valdes AM, Slatkin M, Freimer NB (1993) Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics 133:737–749

Woodroffe R, Ginsberg JR, Macdonald DW (1997) The African wild dog-status survey and conservation action plan. IUCN, Gland