

Reproductive sharing and proximate factors mediating cooperative breeding in the African wild dog (*Lycaon pictus*)

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Abstract Although dominant African wild dogs (*Lycaon pictus*) are generally believed to be the sole breeders within a pack, earlier behavioral and endocrine data suggest that reproduction could be shared with subordinates. We performed an extensive behavioral, demographic, and genetic evaluation of a wild dog population in South Africa to examine the level of such sharing and the proximate mechanisms influencing reproductive contributions of each sex. While a majority of pups were born to dominants because of a lack of subordinate potential breeders, we discovered a substantial portion of reproductive sharing between dominants and subordinates. Compared with alpha females that mated annually, subordinate beta females bred in 54.5% of years whereas thetas never bred. The three top-ranking males all sired pups (56.0%, 32.0%, and 12.0%,

respectively) when three or more adult males were present. With only two pack males, alpha and beta individuals shared reproduction nearly equally (55.2% and 44.8%, respectively), and litters of mixed paternity were discovered on eight of 15 (53.3%) occasions. A skewed adult sex-ratio and frequent alpha mortalities for females and behavioral aggression in males allowed most individuals to attain dominant status in their lifetime, creating a constantly shifting social hierarchy. Genetic parentage results corresponded to reported hormone profiles, suggesting physiological suppression in some lower-ranked individuals of both sexes. Thus, a combination of demographic, behavioral, and hormonal proximate factors mediates reproductive partitioning in wild dogs. We conclude that reproductive sharing can be significant in this species, especially for males that have less robust suppressive mechanisms than females.

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Introduction

Cooperative breeders live in social groups where reproduction is partitioned among members to varying degrees, and individuals other than parents help care for offspring (Faulkes and Bennett 2001). In some such species, such as the naked mole rat (*Heterocephalus glaber*, Clarke and Faulkes 1997), dwarf mongoose (*Helogale parvula*, Rood 1990), and jackal (*Canis mesomelas* and *Canis aureus*, Moehlman 1979), social dominants nearly completely monopolize copulations, and reproduction is rare or nonexistent for subordinate adults, even of prime age.

Other species express intermediate levels of reproductive partitioning, with some subordinates being reproductively successful, as in the Seychelles warbler (*Acrocephalus sechellensis*, Richardson et al. 2001), redfronted lemur (*Eulemur fulvus rufus*, Kappeler and Port 2008), and Jamaican fruit-eating bat (*Artibeus jamaicensis*, Ortega et al. 2008). Still, others express almost equal division in reproductive capacity among group members, as in the coati (*Nasua narica*, Russell 1983) and banded mongoose (*Mungos mungo*, Creel and Waser 1991). This substantive diversity among species suggests that there are complex mechanisms regulating reproductive sharing. Such information is crucial for understanding the processes of sexual selection, reproductive skew, and inclusive fitness. Yet, studies to precisely identify these driving factors are challenging due to the lack of large-scale genetic and behavioral analyses of wild populations that include lineage relationships.

Comprehensive studies reveal that proximate mechanisms of subordinate reproductive suppression may encompass behavioral, physiological, and/or demographic factors (French 1997). Behavioral mechanisms, such as aggression (Reyer et al. 1986), can profoundly influence distribution of reproduction with dominants physically blocking a subordinate's access to mates, as observed in the gray wolf (*Canis lupus*, Packard et al. 1985). Mate guarding is a similar tactic (Birkhead and Moller 1992) used by the mustached tamarin (*Saguinus mystax*, Huck et al. 2004). Physiological suppression by means of stifling a prominent reproductive hormone (i.e., testosterone) has been effective, as seen in the naked mole-rat (Faulkes and Abbott 1991). An indirect strategy may also occur, as when subordinates produce elevated glucocorticoids (stress hormones) that, in turn, compromise normal reproduction, as reported for the song sparrow (*Melospiza melodia*, Wingfield and Silverin 1986). A combination of behavioral and hormonal cues is also well known to limit subordinate reproduction in the common marmoset (*Callithrix jacchus*, Barrett et al. 1993) and cotton-top tamarin (*Saguinus oedipus*, Savage et al. 1988). In the context of demographics, group size and composition are significant. For example, chances for subordinate male savannah baboons (*Papio cynocephalus*, Alberts et al. 2003) to breed are vastly improved in larger troops due to increased energetic costs of mate guarding for dominants. Thus, the mechanisms associated with reproductive contributions appear as diverse as the phenotypes of the cooperatively breeding species studied to-date.

To improve our understanding of the phenomenon of reproductive sharing, we chose the endangered African wild dog (*Lycaon pictus*) as a study species, largely because of its well-recognized, highly cooperative, and complex social system (Estes and Goddard 1967). Although naturally found at low densities, packs as large as 27 individuals

are known to range an average of 12 km daily to hunt prey (Creel and Creel 2002). The standard model of a wild dog pack consists of a dominant breeding pair, several subordinate non-breeding adults (usually siblings of the same-sex dominant individual), and subordinate offspring of the alpha pair (Girman et al. 1997). After a birth (usually once per year in a given pack), all group members cooperate in provisioning the lactating female in the den and feeding/protecting pups after emerging from underground (Malcolm and Marten 1982; Creel and Creel 2002). After 1 year of age, offspring disperse as far as 250 km from the natal pack territory in single-sex, sibling groups (Fuller et al. 1992) in search of opportunities to join other dispersers or an already established group (Frame et al. 1979). Once a new breeding pack forms, a clear social dominance hierarchy develops within each gender, with reproductively “prime”, middle-aged males and the oldest female usually holding dominant positions (Creel and Creel 2002).

The prevailing view has been that dominant female and male wild dogs are the sole breeders within a pack, with most pups resulting from this pairing (Malcolm and Marten 1982; Girman et al. 1997). One study estimated that alpha females and males achieve ~96% of total annual reproductive success through mating and offspring production compared with only ~10% for occasionally breeding subordinates (Girman et al. 1997). However, exceptions to alpha reproductive dominance have been reported, with behavioral observations suggesting that subordinate females have produced 19% of litters in Kruger National Park (South Africa; Reich 1981), 24% in the Selous (Tanzania; Creel and Creel 2002), and 25% in the Serengeti ecosystem (Tanzania; Malcolm and Marten 1982). Only three small-scale genetic analyses have compared reproductive contributions between wild dog social classes. In a study of nine packs in Kruger National Park, Girman et al. (1997) found that subordinate females and males produced only 8% and 10% of pups, respectively. Creel and Creel (2002) examined two litters in the Selous and found one pup in each litter not sired by the alpha male. Moueix (2006) reported that at least one pup in each of five sampled litters from Madikwe Game Reserve and Pilanesburg National Park (South Africa) was not offspring of the alpha male.

We conducted the first large-scale molecular genetics, demographic, and behavioral assessment of a wild dog population in KwaZulu-Natal (KZN) Province, South Africa, in order to answer three important questions about the relationship between social rank and reproductive opportunities in this species. First, how much reproduction is shared between dominants and subordinates in a breeding pack? Second, what behavioral, demographic, or physiological factors are influencing distribution of reproductive opportunities? And third, does the degree of reproductive

sharing or the mechanisms that control reproductive contributions vary between sexes?

We hypothesized that the incidence of shared reproduction among dominant and subordinate African wild dogs was higher than previously reported for this species. We also expected that a combination of demographic, behavioral, and hormonal factors determined the distribution of breeding opportunities and that mechanisms mediating reproductive sharing were different for males and females. To test these assumptions, we evaluated extensive behavioral and genetic data collected through radio-telemetry monitoring, frequent behavioral observations, individual fecal sampling, and occasional field immobilizations. Specifically, we compared social rank and genetic parentage results, examined behavioral processes maintaining dominance hierarchies, compared our extensive genetic results to hormonal data for dominants and subordinates collected earlier by others (Creel et al. 1997), and evaluated demographic processes at work in the population.

Materials and methods

Study population

Demographic and behavioral information was collected on African wild dogs in KZN from January 2001 through August 2008. This population began successfully breeding and expanding in 2001 after the release of artificially assembled packs into a single protected area. By August 2008, further reintroductions, natural dispersals, and pack formations boosted the population to 88 dogs in eight different groups living in three protected areas (Spiering et al. 2009). During the study period, the population included 257 individuals that comprised ten packs and 36 total pack years, with successful breeding occurring in 32 of these years.

Data on pack composition (number of dogs, sex, age classes, and litter size at first emergence from the den), location, and reproductive status (i.e., breeding, non-breeding, pregnant, lactating) were collected once monthly minimally and as often as ten times per month for more accessible packs. Packs and dispersing groups were located by radio-telemetry, observations made from a vehicle or on foot, and individual wild dogs identified by unique coat patterns and photographic records.

Determining dominance

The alpha male and female in a given pack were recognized on the basis of: (1) reciprocal male and female scent-marking behavior (Frame et al. 1979); (2) obvious co-incident male and female movement; and (3) mutual offensive and defensive maneuvers in agonistic encounters

with other adult pack members (Girman et al. 1997). The dominance hierarchy was also inferred from gestures of subordination, including laying the ears flat against the head and/or rotating the head away from a higher ranking individual (van Lawick 1970) as well as passive submission that included a subordinate rolling onto its back in the presence of a more dominant dog (Schenkel 1967).

In this study, we considered three ways for an individual to gain the dominant position within a pack. First, a wild dog could become dominant by default as the only adult of their sex in the pack. Alternatively, there could be inter-animal competition without physical aggression with a same-sex adult at the initial pack-bonding phase or when the hierarchy was disrupted due to death of the alpha individual. Lastly, the current dominant could be overthrown by a competitor through fighting. As dominant females never disperse (Creel et al. 2004), we assumed that any such individuals missing from a pack had died. Subordinate siblings of the alpha pair were considered “potential breeders”, whereas subordinate offspring of the dominant pair were not because alpha individuals apparently share breeding with siblings, but rarely with offspring (Girman et al. 1997; McNutt and Silk 2008; Spiering et al., unpublished observations).

Genetic sampling and genotyping

Biomaterials for molecular genetic evaluations were collected from January 2003 through January 2008. Wild dog tissue and blood samples were obtained opportunistically during immobilization operations for translocation and collaring and when a wild dog carcass was located (Spiering et al. 2009). Non-invasive collection of feces allowed securing representative samples from a significant-sized population ($n=113$ wild dog individuals and ten packs). Fecal samples were collected fresh from known individuals within 5 to 30 min of deposition and then kept in a cool bag for up to 4 h before storing in labeled, plastic freezer bags at -20°C until genetic analysis.

Sample collection and detailed DNA extraction protocols are described in Spiering et al. (2009). In brief, DNA was extracted from scat using a QIAamp DNA Stool Mini Kit and from tissue and blood using a QIAamp Tissue and Blood Kit (QIAGEN). Genetic analyses were completed using 19 microsatellites selected from the 2006 International Society for Animal Genetics domestic dog (*Canis familiaris*) panel that were consistent with other wild dog genetic studies in southern Africa (Moueix 2006). All individuals were genotyped at 17 dinucleotide microsatellite loci (AHT130, AHT137, AHTh171, AHTh260, AHTk211, AHTk253, CXX279, FH2848, INRA21, INU030, INU055, LEI004, REN54P11, REN105L03, REN162C04, REN169D01, and REN247M23) and two

tetranucleotide loci (FH2054 and FH2328). These loci are commonly used for determining parentage in domestic dogs and, therefore, were selected because they are widely distributed throughout the genome and highly polymorphic. The polymerase chain reaction protocols are discussed in Spiering et al. (2009). A combination of the multiple tubes approach (Taberlet et al. 1996) and the maximum likelihood method (Miller et al. 2002) were used to overcome the potential for fecal DNA genotyping errors (Spiering et al. 2009). To detect and eliminate sampling error, we compared matched tissue or blood with feces, analyzed duplicate samples for individuals, and used a significant number of microsatellite markers to verify unique individuals in the dataset (Waits et al. 2001).

Parentage analysis

During the study period, 220 pups emerged from 30 litters, and 86 of these offspring were sampled for parentage analyses using the likelihood-based approach in the Cervus 3.0.3 software package (Marshall et al. 1998). The simulation program in Cervus was used to establish the critical difference in natural logarithm of the odds ratio (LOD score) between the first and second most likely candidate parents (at >95% confidence). Only adults from within the pack with a given set of offspring were considered candidate parents because no extra-group copulations have been reported for this species (and analyses later confirmed that all parentage was assigned to pack members). As most wild dog packs are comprised of a group of brothers and an unrelated group of sisters (Girman et al. 1997), we completed all simulations with and without the advanced simulation option that includes relatives among candidate parents. Assignments using the advanced option with relatives did not differ from assignments not using the function.

A lower than expected frequency of heterozygotes indicating a high frequency of null alleles was detected at locus INU030, which, consequently, was excluded from further analysis. No other locus deviated from Hardy–Weinberg equilibrium. For the 18 loci used, the overall probability of exclusion was 0.991 for the first parent and 0.999 for the second. Critical LOD values were calculated to assign: (1) maternity, with paternity unknown (in cases where multiple females appeared to be pregnant); then (2) paternity, with known maternity (in cases where multiple adult males were present); and (3) the parent pair, with sexes known (to verify assignments). Each breeding pack was simulated and assigned parentage separately, which allowed entering pack-specific data, including the proportions of potential parents sampled and the relatedness between candidate parents. All statistical analyses were performed using JMP software version 3.2.2 (SAS Institute Incorporated) with results presented as means \pm standard error of the mean.

Results

Reproductive sharing

Maternity

The age of first-litter production for a female varied from 1.3 to 5.0 years (mean, 3.2 ± 0.3 years). The number of litters produced per breeding female ranged from one to seven (mean, 2.1 ± 0.4 litters). Alpha females produced 32 litters in 36 breeding years, resulting in an $88.9 \pm 5.3\%$ annual probability of breeding for dominants. In the remaining 4 years, packs formed late in the year or a dominant died, thereby causing reproductive failure. Alpha females gave birth to 93.0% of the 86 pups that were genetically sampled (22 litters), whereas the remaining 7.0% were produced by beta counterparts. However, 68 of the 86 pups (79.1%) whelped by alpha females were born to packs comprised of only one adult female at that time.

When multiple adult females were present in a pack, physical signs of pregnancy (i.e., greatly increased weight, enlarged teats) were never observed in more than two females at one time. Although a few matings were observed in 2 years involving theta females, none subsequently appeared pregnant on the basis of physical appearance or offspring production. Alpha individuals mated every year compared to beta counterparts that bred in 6 of 11 breeding years ($54.5 \pm 15.7\%$; Table 1; Wilcoxon test, $T_{41} = 5.04$, $P < 0.001$). Female subordinates of all ranks gave birth to six litters in 16 individual years, resulting in a $37.5 \pm 12.5\%$ probability of a subordinate breeding. In breeding years, with multiple adult females present, six alpha females whelped 12 sampled pups (66.7%), and three subordinates produced six sampled pups (33.3%; Table 1). There was no significant difference in average number of pups per litter for years with single (7.4 ± 0.6 pups, 26 litters) versus multiple (8.8 ± 1.4 , 3 litters; Wilcoxon test, $T_{27} = 0.80$, $P = 0.43$) births. Although sample size was small, on the three occasions when two litters were living within the same den (i.e., creched), the proportion of emerging pups whelped by the alpha female ($54.1 \pm 21.0\%$) was similar to that for subordinates ($45.9 \pm 20.5\%$; t test, $t_4 = 0.37$, $P = 0.80$). There was also no significant difference in percentage survival to 1 year for pups born to alpha versus beta mothers ($83.4 \pm 16.7\%$ and $100.0 \pm 0.0\%$, respectively; t test, $t_2 = -1.00$, $P = 0.42$).

Paternity

Males first bred from 1.1 to 5.0 years of age (mean, 2.9 ± 0.2 years), which was not significantly different from females (3.2 ± 0.3 years; Wilcoxon test, $T_{31} = 0.78$, $P = 0.44$). During the study interval, individual breeding males produced from one to five litters each (mean, $1.7 \pm$

Table 1 Number of years with multiple African wild dog females pregnant and number of sampled pups (18 pups over 11 pack years) parented by dominant versus subordinate females

Pack	Year	No. of adult females	Multiple pregnant females?	No. alpha female offspring	No. beta female offspring	Observations
Imfolozi	2001	2	No	1	0	
Crocodile	2003	3	Yes	NGD	NGD	Multiple males mated with all females. No pups emerged from den.
	2004	2	No	3	0	Males mated with both females, but no pregnancy in the beta.
Mkhuze	2005	3	No	1	0	Offspring only from the alpha female.
	2006	3	Yes	0	1	Alpha female died before whelping. Beta female whelped ~2 weeks later.
Thanda	2006	2	Yes	3	1	
	2007	2	No	NGD	NGD	Pups and alpha females killed at den.
	2008	3	Yes	NGD	NGD	Alpha and beta female litters killed by lions at den.
Ume	2007	3	Yes	2	4	Beta female whelped ~2 weeks after alpha females.
	2008	2	Yes	NGD	NGD	Large litter of 14 pups emerged. No genetic samples.
Veggie	2007	2	No	2	0	
Mean		2.5	54.5%	66.7%	33.3%	

NGD indicates no genetic data available

0.3 litters). Based on genetic analyses, alpha males were confirmed to have sired 72.1% of pups compared with 22.1% for beta and 5.8% for theta counterparts (Table 2). Alpha males sired offspring in 20 of 24 sampled litters, resulting in an $83.3 \pm 7.7\%$ chance of breeding annually. However, for 32 pups in six litters (37.2% of sampled pups), only one adult male was present and was confirmed to have sired all pups.

Without exception, if present in a pack, at least one subordinate male was observed mating with the alpha female, a subordinate female, or both. In the presence of multiple potentially breeding males, the dominant individual sired 55.5% compared with the beta at 38.9% and theta at 5.6% of all offspring (based on 54 pups in 17 litters; Table 2). When only two adult males were present,

the incidence of reproductive sharing was high, and the percentage of pups sired did not differ significantly between the alpha (55.2%) and beta (44.8%; t test, $t_{14}=0.75$, $P=0.47$) males, with similar annual probabilities of siring offspring (alpha, $80.0 \pm 16.4\%$ versus beta, $60.0 \pm 15.7\%$; $t_{28}=1.18$, $P=0.25$). In the presence of three or more males in a pack, the dominant individual produced 24% more pups than the beta that, in turn, sired 20% more offspring than the theta (Table 2). The overall annual probability of breeding for the alpha males ($86.4 \pm 7.5\%$) was not significantly different than for the beta counterparts ($73.3 \pm 11.8\%$; Wilcoxon test, $T_{35}=0.98$, $P=0.33$), but both were higher than for theta individuals ($28.6 \pm 18.4\%$; $T_{27}=3.45$, $P=0.002$ and $T_{20}=2.09$, $P=0.048$, respectively).

Table 2 Numbers and mean percentages of sampled pups emerging from dens sired by alpha, beta, and theta African wild dog males

	No. pups	No. litters	Alpha male pups (%)	Beta male pups (%)	Theta male pups (%)	Subordinate males total (%)
All sampled pups	86	24	72.1	22.1	5.8	27.9
Alpha male only packs	32	6	100.0	NP	NP	NP
Multiple adult male packs	54	17	55.5	38.9	5.6	44.5
Two adult males	29	9	55.2	44.8	NP	44.8
Adult males ≥ 3	25	8	56.0	32.0	12.0	44.0

NP indicates individual of that rank not present

In 15 breeding years when subordinate males were present, multiple sires fathered pups in eight single litters, with the alpha male siring the majority of three mixed-sire litters. Two males were present in eight breeding years where the dominant individual sired $58.8 \pm 16.5\%$ of pups, which was not significantly different than the $41.3 \pm 16.5\%$ for beta individuals (t test, $t_{14}=0.75$, $P=0.47$). In the seven litters with three potential male breeders, the alpha male sired $55.4 \pm 12.3\%$ of young compared with $30.3 \pm 5.5\%$ and $14.3 \pm 10.2\%$ for the beta and theta individuals, respectively (Table 3). For seven litters and 35 pups, there was no statistically significant difference between the proportion of pups surviving to 1 year that had been sired by dominant ($78.8 \pm 12.9\%$) versus subordinate males ($93.8 \pm 5.0\%$; t test, $t_{13}=-1.14$, $P=0.30$).

Factors mediating reproductive opportunities

Population demographics

Although the average lifespan of African wild dogs in our population was 2.0 ± 0.2 years, dispersing individuals that formed breeding packs lived 4.1 ± 0.3 years, which was similar to the mean of 4.7 ± 0.5 years for animals gaining dominance status (Wilcoxon test, $T_{46}=-0.91$, $P=0.37$). During the breeding season in May, pack size ranged from two to 23 adults and yearlings (mean, 8.1 ± 0.8 individuals/

pack). At emergence from the den, litter size varied from two to 14 pups (mean, 7.6 ± 0.6 pups/litter) with a sex ratio near parity (0.51 ± 0.04). The number of potentially breeding adults (intra-pack adults that were siblings, half-siblings, fathers or uncles, but not offspring of the dominant pair) varied from two to seven (mean, 3.6 ± 0.5 adults/pack). The remainder of each pack (i.e., $54.7 \pm 6.5\%$ of individuals) was comprised of offspring from the alpha pair, generally considered to be non-breeders and confirmed as such in this study. As observed in other wild dog populations (McNutt and Silk 2008), adult and yearling males outnumbered females slightly but not significantly within packs (males, $54.9 \pm 3.9\%$ versus females, $45.1 \pm 2.6\%$; t test, $t_{62}=-1.13$, $P=0.21$). However, this pattern was significant when considering only potentially breeding adults (males, $59.7 \pm 3.2\%$ versus females, $40.3 \pm 5.8\%$; $t_{62}=-3.19$, $P=0.003$). Of the 32 successful breeding years evaluated, only 11 (34.4%) were years when more than one adult female potential breeder was in the pack (i.e., generally there was a lack of subordinate females available for breeding). In contrast, multiple adult males were present in 22 (68.9%) of these same breeding years.

Influence of demographics and behavior on rank

Only 25.3% of the wild dogs in our study population survived to disperse from the natal group and form a breeding pack. An

Table 3 Percentages of litters sired by alpha, beta, and theta African wild dog males in 15 breeding years with subordinate male potential breeders present. Numbers represent percentages of pups sired within single litters

Pack	Year (no. of litters)	No. subordinate males	No. sampled pups	Alpha male offspring (%)	Beta male offspring (%)	Theta male offspring (%)
Imfolozi	2001 (1)	1	1	100.0	0	NP
	2002 (1)	1	5	100.0	0	NP
	2003 (1)	1	10	20.0	80.0	NP
	2004 (1)	1	3	100.0	0	NP
Crocodile	2004 (1)	2	3	33.3	33.3	33.3
	2005 (1)	2	3	0	33.3	66.7
	2006 (1)	2	7	71.4	28.6	0
	2007 (1)	1	3	100.0	0	NP
Juma	2005 (1)	1	2	0	100.0	NP
	2006 (1)	1	1	0	100.0	NP
	2007 (1)	4	3	66.7	33.3	0
Mkhuze	2005 (1)	3	1	100.0	0	0
Thanda	2006 (2)	1	4	50.0	50.0	NP
Ume	2007 (2)	2	6	66.7	33.3	0
Veggie	2007 (1)	2	2	50.0	50.0	0
Total pups			54	30	21	3
Mean % of litters with two adult males in pack				58.8	41.3	NP
Mean % of litters with ≥ 3 adult males in pack				55.4	30.3	14.3

NP indicates individual of that rank not present

even smaller percentage (13.3%) became dominant in the pack during their lifetime, which was similar to the proportion of dogs that eventually reproduced (15.2%). Of all dogs surviving dispersal and living in a breeding pack for at least 1 year, 76.5% eventually became dominant.

On average, dominance tenures lasted 2.4 ± 0.3 years, with alpha females tending to have non-significantly longer such periods (2.7 ± 0.5 years) than males (2.1 ± 0.4 years; t test, $t_{38} = 0.17$, $P = 0.86$). Of the packs having multiple, potentially breeding adult males ($n = 8$ packs, 19 pack years), there was major variation in the frequency of alpha male replacement. One pack remained stable for more than 2 years, one changed dominants randomly three times in 4 years, and others experienced dominance switches annually or biannually. Within packs with multiple females ($n = 7$ packs, 14 pack years), alpha females were sustained in multiple years for three packs, but, in all other cases, dominants changed annually or biannually, usually as a result of alpha female mortality. In years with multiple, potentially breeding adults, male dominance changes occurred in $65.6 \pm 5.3\%$ of pack years compared with $41.7 \pm 4.1\%$ for females (Wilcoxon test, $T_{31} = 1.15$, $P = 0.31$). Although rates of hierarchical change were similar between sexes, the causes leading to reestablishment of social dominance roles were different. Changes in dominance status were more likely to occur as a result of aggression in males than in females (males, 45% versus females, 5%; Fig. 1; t test, $t_{38} = -3.21$, $P = 0.003$). In contrast, females most often earned dominance via non-aggressive competition at pack formation, after the death of an alpha individual, or by default as being the sole surviving adult female in the pack (Fig. 1). A higher probability of achieving social dominance was observed with increasing age, as all males and females in our population surviving to 6 years of age or older were dominant. In each case, these dogs were the sole individual of their sex left in the pack and, therefore, attained dominance (and became the only reproducers) by simply outlasting competitors.

Discussion

Results from our integrated demographic, behavioral, and genetic analysis of social dominance and parentage in a

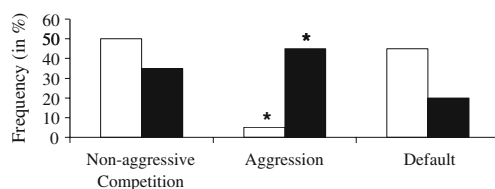


Fig. 1 Frequency distribution of the mechanisms used to achieve dominance for 20 female (open bars) and 20 male (solid bars) African wild dogs. An asterisk indicates a significant difference ($P = 0.003$)

multi-pack population of African wild dogs supported the hypothesis that reproduction was shared among adults substantially more than previously reported. While an earlier behavior-genetics study reported that lower-ranking individuals rarely bred (subordinate females and males producing only 8% and 10% of offspring, respectively; Girman et al. 1997), our detailed genetic testing revealed significant reproductive sharing with, and pup production by, subordinate adults, especially males. In the presence of multiple adult potential breeders, we found that subordinate females whelped 33.3% and subordinate males sired 44.5% of pups. Subordinate females became pregnant in 37.5% of individual years with beta individuals reproductively successful in 54.5% of years. It is unclear if the litters sampled by Girman et al. (1997) were born into packs with several potential breeders or if packs consisted only of a single alpha pair and their offspring. This would greatly alter results of subordinate breeding, as sharing reproduction has been reported with siblings of the alpha pair but never with adult offspring. When both single alpha pair packs and multiple potential breeder packs were considered, our results revealed female percentages (7.0%) similar to Girman et al. (1997), but subordinate male reproduction was still substantially higher (27.9%).

Contrary to a previous investigation suggesting that male and female subordinates reproduce at similarly low levels (Girman et al. 1997), our study confirmed that the proportion of subordinates able to breed and produce offspring was higher for males than females. This observation corroborated earlier speculation by Creel et al. (1997) who, in interpreting gonadal hormone profiles, predicted that shared paternity should be more common than shared maternity in the African wild dog. We determined that female reproduction was only shared with the beta female regardless of number of available female potential breeders. In contrast, when there were three or more adult males in a pack, each almost always sired some pups in a given litter. Reproductive partitioning extended as far as the third ranking male, a mechanism absent in females where the third position apparently offers no direct reproductive benefit.

Our discovery of shared reproduction among pack members, even including multiple sires in a given litter, suggests that this strategy contributes to ensuring the fitness of the pack and gene diversity in progeny (Gottelli et al. 2007). This is advantageous in an unpredictable environment or when the risk of disease-related mortality is high (Sherman et al. 1998). Others have maintained that increased genetic variation could simply be the result of multiple matings rather than its selective force (Wolff and Macdonald 2004). The wild dog may have also evolved a multiple mating strategy to promote post-copulatory sperm competition (Madsen et al. 1992) or to encourage all males to contribute equally to caring for young (Nakamura 1998).

Our results revealed that a combination of factors appeared to mediate reproductive partitioning within the African wild dog breeding pack. Within the ecological conditions of KwaZulu-Natal, both demographic and behavioral mechanisms created a constantly shifting dominance hierarchy and social system. Although genetic analyses confirmed that dominants indeed parented the majority of offspring, we found that alpha male and female dominance tenures were relatively short, with frequent within-pack hierarchical changes that permitted multiple adults to gain top-tier status and reproduce. Although only ~25% of African wild dogs born into this population survived long enough to join a breeding pack, 75% of those succeeding at this endeavor eventually became alpha. The slightly longer (non-significant) dominance tenures for females compared with males was similar to observations of Creel et al. (1997), but there also was substantial inter-pack variation in dominance stability for both sexes. Regardless, we were most impressed with the overall short lifespan of the average wild dog in breeding packs (ca. 4 years) that, in turn, stimulated a constantly changing society where frequent alpha deaths provoked dominance changes, ranging from inheritance of dominance to dispersals to search for new mates. The incidence of mortalities measured in our study was similar to that reported by Woodroffe et al. (2007) for other wild dog populations in Africa. Therefore, it is reasonable to suspect that a continually changing hierarchical social system is a common trait of this species regardless of regional location.

The basis for abbreviated dominance tenures was markedly different between males and females, with the former changing via aggression and the latter mostly by nonviolent competition at pack-bonding. Whereas there was little physical antagonism among females (regardless of social status), dominant males often reaffirmed dominance by aggression with subordinates frequently challenging alpha male status. Both genders displayed the capacity to advance their status opportunistically upon the death of an alpha individual. Exactly how a wild dog individual secures the advantage to win dominance in these situations is unknown and is fascinating given that most competitive interactions often were between closely related and physically-similar individuals (i.e., siblings). More study is warranted, especially exploring the potential of mate choice by either sex in influencing who attains the alpha (and breeding) positions.

It was clear from our investigation that a female often achieved dominance by default when she alone joined a male dispersal group or when the death of a dominant sibling resulted in her being the sole surviving adult female in the pack. Our results supported the findings of Creel and Creel (2002) and Somers et al. (2008) that the probability of being socially dominant increases with female age, as all

females in our population surviving to 6 years or older became dominant. In fact, there was an overall skewing of sex ratio for mature wild dogs to favor males (60%) despite there being near-gender parity in the pup age class. Frequent adult mortalities led to a prevalence of packs with only a single female, with multiple adult females occurring during only about one third of all pack breeding years. This was not surprising given the findings of others (Frame et al. 1979; Reich 1981; Creel and Creel 2002) who have noted progressively increasing male bias in older age classes. In the KZN population, generally, males had more contemporaries than females in a breeding pack and, thus, more competitors that decreased the chance of securing dominance after the death of an alpha male. This explained why only 20% of males achieved dominance by default and rather relied on constantly striving to win dominance by aggressively and/or competitively overthrowing siblings. In contrast, females experienced less competition within the pack and perhaps developed a patient “waiting” strategy, seeking out active reproductive opportunity only after the alpha female's death. Interestingly, subordinate males reproduced more than subordinate females, indicating that more competitors were not necessarily a significant obstacle to males achieving reproductive success.

When breeding packs did contain multiple adult females, reproductive success of subordinates was noticeably higher than reported by Creel and Creel (2002), probably because our population often was comprised of newly formed packs with less stability in dominance hierarchies. In three of 11 breeding years, packs with multiple female potential breeders had formed shortly before the breeding season, and, on many occasions, copulations were observed between several males and females before pack dominance hierarchy was established. This is not unusual as others have observed multiple females and males copulating in earlier African wild dog studies (Malcolm and Marten 1982; Girman et al. 1997). Occasionally, this has resulted in births to subordinate females with the litters then sometimes killed by the alpha female (Reich 1981; Malcolm and Marten 1982; Fuller et al. 1992). Although our sample size for these genetic comparisons was modest, we observed no infanticide on the three occasions where two litters were produced in a pack simultaneously, and similar pup numbers were whelped by the alpha and beta females.

Our study is the first to show a parallel between the extent of reproductive sharing and behavioral and endocrine correlates of rank reported for the African wild dog in the Selous (Creel et al. 1997). For instance, Creel et al. (1997) found that female dominant African wild dogs excreted higher estrogen and progesterin concentrations in feces and were more aggressive during mating periods than all subordinates. Although it was predicted that subordinate females in the Selous did not ovulate due to high baseline

estrogens and estrogen-to-progesterone ratios (Creel et al. 1997), we suspect that most beta females in our population completed a normal ovarian cycle, in part because at least five of these individuals actually produced pups. However, this ovarian activity likely was temporally delayed based on betas always appearing visibly pregnant later and whelping pups 2 to 4 weeks after the alpha. Since no females that ranked third or lower in the dominance hierarchy became pregnant in our population, it was possible that these individuals were physiologically suppressed and failed to ovulate. This seemed especially likely as little aggression was observed between females to suggest behavioral mechanisms of limiting lower ranking individuals' access to males. Creel et al. (1997) also reported differences in androgen excretion among males in the hierarchy, the highest values being in the top-tier dog, which was believed to present advantages in reproductive capability and aggression. Our results, however, demonstrated clearly that beta and theta males were physiologically capable of siring young, but differences in gonadal steroid production may have affected fertility in males ranking third and lower. Nonetheless, most reproductive benefits probably are conferred behaviorally by dominants being more likely to block access to females in estrus through enhanced antagonistic, female guarding activities.

In conclusion, our study has demonstrated that reproductive success of the African wild dog is influenced by (1) a continuously dynamic social system where marked hierarchical shifts ensure that a diversity of individuals eventually become alpha reproducers and (2) a greater than previously reported incidence of reproductive sharing, especially involving subordinate males. Both beta males and females played a significant role in producing viable young that appeared as robust as those from the dominant pair. Although the alpha position was considered most attractive to all pack members, this top-tier status was not necessary to contribute offspring. Nevertheless, short life spans and an aspiration to be dominant appeared to drive a constantly shifting social order, with males mainly relying on behavioral aggression and females remaining vigilant for nonviolent opportunities to escape possible hormonal suppression. We suspect that this strategy assists in sustaining genetic diversity and more effectively maintains heterogeneity than in other cooperative breeders (i.e., naked mole rat, Clarke and Faulkes 1997; dwarf mongoose, Rood 1990), where reproduction is almost exclusively monopolized by alpha individuals. This is not the case in the African wild dog where more individuals are contributing genes to offspring, even when only a single litter is produced annually.

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