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A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*)

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Abstract The African wild dog is a highly social, pack-living predator of the African woodland and savannah. The archetypal wild dog pack consists of a single dominant breeding pair, their offspring, and non-breeding adults who are either offspring or siblings of one of the breeding pair. Non-breeding adults cooperate in hunting, provisioning and the protection of young. From these observations follows the prediction that the genetic structure of wild dogs packs should resemble that of a multigenerational family, with all same-sexed adults and offspring within a pack related as sibs or half-sibs. Additionally, a higher kinship between females from neighboring packs should be evident if females tend to have small dispersal distances relative to males. We test these predictions through analysis of mitochondrial DNA control region sequences and 14 microsatellite loci in nine wild dog packs from Kruger National Park, Republic of South Africa. We show that as predicted, African wild dog packs generally consist of an unrelated alpha male and female, subdominant close relatives, and offspring of the breeding pair. Sub-dominant wild dogs occasionally reproduce but their offspring rarely survive to 1 year of age. Relatedness influences the timing and location of dispersal events as dispersal events frequently coincide with a change in pack dominance hierarchy and dispersers often move to areas with a high proportion of close relatives.

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Introduction

The African wild dog is known for a well developed, highly cooperative social system (Estes and Goddard 1967). Wild dog packs generally consist of a dominant breeding pair, several subdominant non-breeding adults, and yearlings, all of whom cooperate to provision the alpha female and her pups. Males often remain in their natal pack whereas sibling female groups emigrate to form new packs with unrelated males (Estes 1991; Kingdon 1977). This model of wild dog social organization is based primarily on studies of packs from the Serengeti Plains in northern Tanzania (van Lawick-Goodall and van Lawick-Goodall 1970; Schaller 1972; van Lawick 1974; Frame and Frame 1976; Frame et al. 1979; Malcolm and Marten 1982) and the research of Reich (1978, 1981) in Kruger National Park, the Republic of South Africa.

In this paper, we use molecular genetic data to test several specific hypotheses and predictions that follow from observations of wild dog social structure (Table 1). We examined 92 individuals from nine packs in the Kruger National Park, the Republic of South Africa from 1989 to 1994. First, because the dominant alpha male and alpha female are thought to be the primary, and generally the only, breeders within the pack, the majority of pups should be their offspring. However, exceptions to reproductive dominance by the alpha pair have been reported. Subdominant males have been observed to copulate with the alpha female, although the number of offspring from subdominant males that survive to reproductive age, if any, is not known (Frame et al. 1979). In cases where subdominant females have produced litters, competition for resources was severe and the alpha female interfered with the provisioning of the subdominant's pups during the first year of devel-

Table 1 Hypotheses regarding the social structure of African wild dogs that were examined through the analyses of mtDNA sequences and 14 microsatellite loci

Hypothesis	Predictions
I. *Parentage*	
A. Not all of the offspring are derived from the alpha male.	Some pups excluded from being offspring of alpha male.
B. Only the offspring of the alpha female survive to one year of age.	Only the alpha female is consistent as mother of one year or greater aged offspring.
II. *Relatedness within Packs*	
A. Adult females in a pack are generally highly related.	Estimated relatedness values are high for pairwise comparisons of adult females in the same pack.
B. Adult males in a pack are generally highly related.	Estimated relatedness values are high for pairwise comparisons of adult males in the same pack.
C. The alpha pair is unrelated.	Estimated relatedness values are low for pairwise comparisons of alpha pairs.
III. *Reproduction*	
A. Subdominants rarely reproduce.	Subdominats are excluded as parents of pack offspring.
B. Similar dispersal frequencies reflect equivalent reproduction of the sexes in each dominance class.	Average proportion of pups produced by males and females is similar for each dominance class.
IV. *Dispersal Distance and Population Substructure*	
A. Female offspring disperse to territories neighboring their natal pack.	Number of related female neighbors is significantly greater than that of a random distribution of dyads.
B. Male offspring do not disperse to territories neighboring their natal pack.	Number of related male neighbors not significantly more than that of a random distribution of dyads.
C. The microgeogeographic substructure of males and females is similar in Kruger National Park.	The distribution of mtDNA genotypes or microsatellite alleles is similar for males and females.

opment (Frame et al. 1979; Kuhme 1965). Thus, if the alpha female influences the survivorship of subdominant young, we would predict that her pups are much more likely to reach 1 year of age. In contrast, the alpha male cannot discriminate between his offspring and those of a subdominant male; thus offspring of a subdominant male should be more likely to survive to reproductive age than those of a subdominant female (Table 1, IA, B).

Second, all adult subdominant pack members are generally thought to be related to one or both of the alpha pair. These subdominant adults cooperate in hunting and in provisioning and protecting the offspring of the dominant pair (Estes and Goddard 1967; Malcolm and Marten 1982). Older adult males are generally brothers of the alpha male whereas older adult females are sisters of the alpha female (Frame et al. 1979). Thus, the persistence of subdominant adults in the pack and their cooperative behavior in rearing the pups is thought to be maintained by the advantages of group hunting and indirect reproductive benefits (Creel and Waser 1992; Estes and Goddard 1967; Hamilton 1964; Malcolm and Marten 1982; Queller 1992; Vehrencamp 1983). In contrast to the high levels of relatedness among individuals of the same sex in each pack, the alpha male and the alpha female are thought to be unrelated (Frame and Frame 1976; Frame et al. 1979). Therefore, we would predict that any two founding adults in a pack who are of opposite sex, and were not the offspring of the alpha pair, should be relatively unrelated, whereas any two adult individuals of the same sex in a pack will be highly related (Table 1, IIA–C).

Third, inbreeding and the potential negative effects of inbreeding depression are thought to be avoided by the observed dispersal of subdominants (Frame et al. 1979; Laikre and Ryman 1991; Noble et al. 1990; Ralls et al. 1986, 1988). Previous studies have suggested that the movement of females is the primary means of genetic exchange among wild dog packs in the Serengeti (Frame and Frame 1976; Frame et al. 1979). One reason for higher levels of female emigration may be the relatively long reproductive tenure of alpha females and the consequent lack of reproductive opportunities for subdominant females (Malcolm and Marten 1982). In contrast, subdominant males may achieve some direct reproductive success through occasional sexual access to the alpha female (Frame et al. 1979). Thus, subdominant females should have less reproductive success than subdominant males. However, more recent studies of dispersal patterns in wild dogs have suggested that the differences in emigration frequency between males and females are not so pronounced. A summary of dispersal data from populations in the Masai Mara, Kenya and Kruger National Park provided evidence of nearly equivalent dispersal frequencies (Fuller et al. 1992a). Conceivably, in these populations, the potential disparity in relative reproductive success between males and females may be less marked than in the Serengeti or reproductive success is not the most significant influence on dispersal patterns. If the former is true in Kruger Park, then we predict similar levels of reproduction by males and females within each dominance class (Table 1, IIIA, B).

Lastly, dispersal distance is thought to differ between males and females. Females generally emigrate to form

packs within the same region as their natal pack (Frame and Frame 1976). Although males have been shown to emigrate less often in the Serengeti, they dispersed greater distances, often to regions distant from their natal pack (Frame et al. 1979). If this disparity in dispersal distance of the sexes is common then we would predict that neighboring packs should include a relatively greater number of highly related female/female pairs than male/male pairs (e.g., Lehman et al. 1992). In addition, we would expect to find greater microgeographic structure among females compared to that of males in Kruger Park (e.g., Melnick and Hoelzer 1992; Table 1, IVA–C).

To test these hypotheses and more accurately assess the role of kinship in determining patterns of social behavior, we applied molecular genetic techniques (Keane et al. 1994; Lehman et al. 1992; Packer et al. 1991; Queller et al. 1993). Potentially the most useful molecular genetic markers for studying the patterns of relatedness within populations are mitochondrial DNA (mtDNA) control region sequences and hypervariable dinucleotide repeat loci (microsatellites) (Ashley et al. 1990; Edwards 1993; Morin et al. 1994a,b; Queller et al. 1993; Roy et al. 1994). Mitochondrial control region sequences have been used in studies at the population level because they have a relatively high mutation rate and do not undergo recombination (Avise et al. 1987; Avise 1992; Brown 1986; Edwards 1993; Patton et al. 1994). However, because mitochondrial DNA is inherited maternally, mitochondrial DNA polymorphisms can only be used to follow female reproductive patterns. In addition, although the mtDNA control region is relatively variable compared to most other known genetic markers, it still may not be variable enough to investigate relationships among members of packs in a single population. Consequently, highly variable nuclear DNA polymorphisms are necessary to assess relatedness among individuals and to measure the contribution of males in a population to subsequent generations.

Microsatellite loci consist of a variable number of tandem repeats of short sequences and evolve through the gain or loss of repeat units rather than sequence substitutions. Because microsatellite loci have mutation rates as high as 1×10^{-3} per gamete per generation, they allow relatedness among individuals in a population to be estimated and permit putative parents to be excluded with a high degree of certainty (Bruford and Wayne 1993; Hughes et al. 1993; Queller and Goodnight 1989; Queller et al. 1993). The microsatellite data were first used to assess the reproductive contribution of alpha males and alpha females through parental exclusion analysis and then to determine whether all subdominant individuals are highly related to one or both of the dominant individuals by estimating relatedness. Similarly, we tested the hypothesis that individuals forming the alpha pair were unrelated. We then estimated the direct reproductive success of dominant individuals as compared to subdominant individuals of both sexes. Finally, dispersal patterns were revealed by utilizing data from mtDNA control region sequences and microsatellite loci. We used these results to test the hypothesis that female wild dogs tend to disperse to territories within or adjacent to their natal pack's home range whereas males tend to disperse to territories that do not border their natal pack. In addition, microsatellite and mtDNA sequence data were used to document differences in the microgeographic structure of male and female wild dogs of Kruger National Park.

Methods

Sampling design

We collected skin or blood samples between 1989 and 1994 from 92 African wild dogs from nine packs in the southern district of Kruger National Park. The number of individuals sampled from each pack varied from 1 to 24 and all individuals were at least one year of age (Fig. 1). Blood samples were obtained from anaesthetised dogs and skin samples by biopsy dart (Karesh et al. 1989). Behavioral data on the packs were also recorded during this period (Maddock and Mills 1994; M. Mills, unpublished work). Wild dogs were individually identified by their coat patterns (Maddock and Mills 1994) and all individuals over 1 year of age in the study population were photographed. Data on the presence of individuals within each pack, their dominant or subdominant status, sex, and location of the pack within the park were usually recorded once per month and often more frequently. Changes in

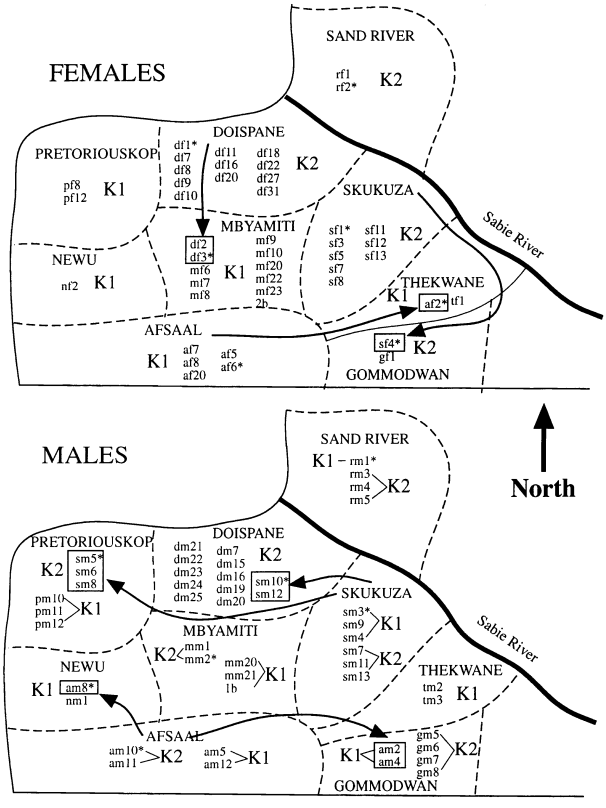

Fig. 1 Location of pack territories and females (*top*) and males (*bottom*) genetically sampled during 1989–1994 in Kruger National Park, Republic of South Africa. Alpha individuals are identified by an *asterisk* and mtDNA genotypes (K1 and K2) are indicated. Known dispersal events by individuals in boxes are depicted by *arrows*

pack range, social hierarchy, and pack membership were infrequent, so monthly monitoring was sufficient to assess change in these parameters. Packs were located by both aerial and ground tracking of radio-collared wild dogs.

Identification of alpha individuals

The alpha male and female in each pack were identified based on the following characteristics: (1) tandem scent marking behavior, defined as reciprocal male and female urination (Frame et al. 1979); (2) coincident male and female movements; and (3) dominance and mutual offense and defense in agonistic encounters with other adult pack members. However, most packs contained several adult males and conclusions regarding alpha status were not made for 6 of 15 litters.

At dens, the presence of lactating females was taken as an indication that they had given birth. We concluded that two females had reproduced if there was morphological evidence of pregnancy in two females followed by a clear bimodal distribution in pub size and/or if the females used different dens. However, in cases where the pups were kept in the same den it was difficult to behaviorally allocate pups to specific mothers because of allosuckling. At one den (Doispane 94, Table 2a) there were three lactating females, but we were unable to ascertain whether all three had given birth due to a lack of genetic samples.

Extraction of DNA

For blood samples, we extracted DNA by proteinase K digestion followed by isolation of the DNA with phenol/chloroform/isoamyl alcohol (PCI) (Sambrook et al. 1989). Alternatively, for skin samples and for blood that did not yield sufficient DNA using the PCI-based protocol, we homogenized the sample with sterile equipment and digested it in 8 mls of TNE (10 mmol L^{-1} TRIS – pH 8.0, 2 mmol L^{-1} EDTA, and 10 mmol L^{-1} NaCL) with 4 mg collagenase, 4 mg proteinase K, 80 mg DTT, and 880 ul 10% SDS for 20 hours at 37° C. The DNA was then isolated by guanidium thiocyanate/silica extractions as described in Boom et al. (1990).

Control region sequencing

The polymerase chain reaction (PCR) was used to isolate and amplify a 402 bp region of the control region of the mitochondrial genome (Saiki et al. 1980). Primers were based on those presented in Kocher et al. (1989; ThrL 5′-CGAAGCTTGATATGAAAAACCATC-3′) and a consensus sequence of human, mouse and cow (DLH-5′-CCTGAAGTAGGAACCAGATG-3′). Double-stranded sequences were amplified in a PCR reaction containing approximately 10 ng of genomic DNA; a reaction buffer of 50 mM KCl, 2.0 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} Tris HCl (pH 8.8), 200 μM dNTP mix, 2.5–12.5 units of *Taq* DNA polymerase, and 25 pmol of each primer in a volume of 50 μl. A Perkin-Elmer Cetus 9600 DNA thermocycler was programmed for 35 to 40 amplification cycles with denaturation at 94° C for 25 s, annealing at 45°–55° C for 30 s and extension at 72° C for 45 s. The DNA was isolated on a 2% low-melting-point agarose gel, recovered using a Gene Clean Kit (Bio 101), and sequenced using a variation of the dideoxynucleotide chain termination method for double stranded sequencing (Sanger et al. 1977; Winship 1989) and a Sequence kit (US Biochemical).

Single stranded conformation polymorphisms

Two internal canid-specific primers were designed from a consensus of six different African wild dog sequences and a gray wolf (*Canis lupus*) sequence: CDLH 5′-CCCTTATTGGACTAGGTGATATGCAT-3′ and LDLL 5′-CCCCTATGTACGTCGTGCATT-3′. Following the initial sequencing of wild dog samples collected from locations throughout their range, the remaining samples were rapidly screened through the analysis of single stranded conformation polymorphisms (SSCP) (Lessa and Applebaum 1993; Girman 1996). Both primers were end-labelled with ^{32}P g-ATP in 25 μl polynucleotide kinase reaction. The end labelled primers were then included in a PCR reaction identical to that used in the sequencing reaction. The PCR products were added in a 1:5 ratio with a 98% formamide stop solution, denatured at 84° C for 5 min and loaded onto a 6% non-denaturing polyacrylamide gel. Products were electrophoresed for 3 h at 40 W such that the gel temperature remains approximately 10° C. Genotype standards representing each control region sequence found in an initial survey were included on every gel. The gels were then dried and exposed to autoradiographic film (Kodak Biomax) for 6–18 h. Several individuals from each SSCP gel were directly sequenced to confirm genotype scores.

Microsatellite analysis

We screened wild dog samples collected from eastern and southern Africa for variation in 60 $CA_{(n)}$ microsatellite loci, originally isolated from a domestic dog genomic library (Ostrander et al. 1993). We identified 14 microsatellite loci that consistently gave PCR product, were polymorphic in wild dogs, and had low levels of polymerase stutter. Detection of microsatellite alleles from genomic DNA was achieved by end-labelling one primer by a standard [g-P^{32}] ATP (Amersham) and T_4 polynucleotide kinase reaction (Sambrook et al. 1989) and performing 28 cycles of PCR amplification in a 25 μl reaction volume using 50 ng of target DNA, 2 mmol L^{-1} $MgCl_2$ and 0.8 μ *Taq* DNA polymerase (Promega). We visualized single stranded alleles by fractionating heat denatured (94° C for 5 min) PCR products onto a 6% sequencing gel containing 50% w/v urea. An M13 control sequencing reaction was run adjacent to the samples to provide an absolute size marker for the microsatellite alleles. Because absolute allele sizes were determined with reference to a size marker we could combine data for different gels.

The Queller and Goodnight (1989) index of relatedness (R) was used to estimate kinship. This index weights each allele by its frequency in the population, so rare alleles are given a relatively higher weight. If a sample adequately represents a population in a Hardy-Weinberg equilibrium, the index values for parent-offspring or full sibling relationship should approach 0.5. Overall, the index values vary between −1 and 1. The Queller and Goodnight (1989) index of relatedness of two individuals (dyads) is defined as:

$$\frac{\sum\sum(Py - P^*)}{\sum\sum(Px - P^*)}$$

where P^* is the population frequency of each allele excluding the compared individuals and Px and Py are the frequencies of each allele in the compared individuals, respectively (e.g., 0.5 or 1 depending on whether the individual is a heterozygote or homozygote). This index is not symmetric, thus reciprocal comparisons are not expected to equal each other (Py over Px). To accommodate for this discrepancy, we calculated the denominator values and numerator values for each of the combinations (Py over Px, and Px over Py), and summed these prior to the division. This procedure would yield an average estimate of relatedness between individuals. The standard deviation of relatedness values were estimated by jackknifing over all loci (Queller and Goodnight 1989).

We estimated the number of loci needed to provide consistent estimates of relatedness by rarefaction analysis. We selected a locus at random, calculated the relatedness, and selected another locus without replacement and recalculated the relatedness based on both loci. The number of loci was increased by addition without replacement until all fourteen loci were selected. We then expressed the difference between consecutive samplings as a function of the total number of loci drawn (Fig. 2). We repeated this procedure 1000 times and calculated mean difference values.

Additionally, we tested whether individuals within packs were more closely related to each other than to individuals from other packs by using Monte Carlo simulations. We compared observed relatedness values for dyads within packs to those calculated from random assignment of individuals to packs, keeping pack sizes and sex ratios constant. Similarly, we determined whether individuals in

neighboring packs were more closely related to each other than expected if individuals were distributed randomly.

To verify parentage, we identified alleles that excluded individuals as the parent of sampled offspring. We calculated the exclusion probability per locus (PE_i) following Chakraborty et al. (1988):

$$PE_i = (1 - \partial - \beta)$$

with ∂ and β are the frequencies of the alleles found in offspring. The probabilities for all loci (Chakraborty et al. 1988; Morin et al. 1994b) were combined as:

$$PE(C) = 1 - \Pi(1 - PE_i)$$

yielding the percentage of randomly chosen adults in the population that could be expected to be genetically excluded as being the father or mother for a given offspring when the other parent is unknown. As discussed by Chakraborty et al. (1988), if potential parents are related, such as might occur in a wild dog pack with related subdominants, the exclusion probability will be an overestimate. We designed a computer program to calculate the number of excluding alleles for all possible parent combinations of each offspring from the entire population. Parental pairs were identified as uniquely having no excluding alleles. In some cases, however, more than two parents were possible and additional behavioral and demographic information (e.g., estimated age or presence in the study population during the breeding season) was used to further verify parentage.

We utilized the results of the paternity analysis to classify dyads as parent/offspring, siblings, and half sibs. The average Queller and Goodnight relatedness values were calculated for each of these groupings and unknown dyads were assigned to the relatedness category whose mean was within one standard deviation of their Queller and Goodnight relatedness values. A few dyads were classified as sibling or parent/offspring although relatedness values were less than one standard deviation from the sibling or parent-offspring mean. In these dyads, a sibling or parent/offspring relationship was suspected based on the exclusion of all other putative parents in the population and their being raised in the same den by a single mother.

We used multi-dimensional scaling (MDS) to summarize allele frequency variation over all 14 microsatellite loci for males and females separately. Monotonic Kruskal's MDS was used because it effectively summarizes allele frequency variation on two dimensions and makes minimal assumptions about the distribution of the data (Borg 1981). A Pearson correlation matrix of allele frequencies was used as the initial input data. The fit of the data to the model was estimated through a Shepard diagram (Shepard 1962), and by the stress factor. The stress factor is a measure of the fit of the data into two dimensions and it varies between zero and one, with values near zero indicating a better fit. In our analysis, the stress factor for both males and females was lower than 0.1 (0.071 and 0.086, respectively). The program SYSTAT for the Apple Macintosh (Wilkinson et al. 1992) was used for these calculations.

Results

Calibration of relatedness estimates

The Queller and Goodnight relatedness index changed little after eight or nine loci were sampled (Fig. 2). Therefore, the inclusion of more than 14 loci in the analysis would not appreciably change our estimates of relatedness. The Kruger Park population was in Hardy-Weinberg equilibrium for all loci except locus 263, which showed an excess of heterozygotes suggesting null alleles were not common enough to cause an appreciable increase in homozygosity above the expected value. In addition, comparisons of known mother/offspring dyads did not reveal the presence of non-amplifying alleles (Pemberton et al. 1995). Finally, the F_{is} pooled over all 14 loci was negative (−0.021), suggesting the population was not inbred.

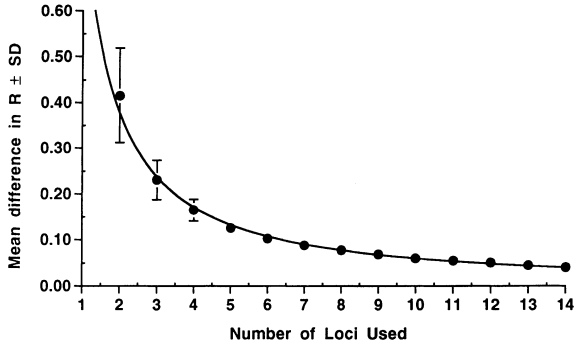

Fig. 2 The relationship of number of loci used to the mean difference between consecutive relatedness (R) estimates. The curve is described by the relation mean difference = 0.831(number of loci)$^{-1.41}$, r = 0.998

To assess whether our data from 14 loci can be used to make estimations of relatedness between individuals of unknown relatedness, we compared the Queller and Goodnight estimates to values for dyads of known relatedness (r). Pairs were identified as mother/offspring or father/offspring based on the absence of allelic exclusions (see methods) and behavioral observations. Pairs identified as siblings were members of a single litter from a single pair of parents determined by exclusion analysis. Similarly, pairs identified as half siblings were determined to have a shared mother but have different fathers. A comparison of known fathers to their offspirng ($r = 0.5$) yielded an average Queller and Goodnight relatedness estimate of 0.51 ± 0.04 whereas a comparison of known mothers to their offspring ($r = 0.5$) showed an average estimate of 0.42 ± 0.05 (Fig. 3). Comparisons of known sibling pairs ($r = 0.5$) and known half-sibling pairs ($r = 0.25$) gave relatedness estimates of 0.47 ± 0.05 and 0.19 ± 0.05 respectively. Comparisons of individuals thought to be unrelated consisted of individuals from the Kruger population compared to individuals from a separate population in the Moremi Game Preserve in Botswana and had an average R of -0.08 ± 0.06. Consequently, within two standard deviations, the Queller and Goodnight estimate of relatedness agrees with the known relatedness values. This close relationship justifies our use of the Queller and Goodnight relatedness estimate to classify dyads of unknown or uncertain relationship.

Parentage

Analysis of microsatellite alleles in all possible fathers revealed that only three of 29 pups (10%) from two different packs, did not have the alpha male as father (Table 2a). The father/offspring random exclusion probability [PE(C)] for all 14 loci combined was 0.996.

Table 2 Number of sampled pups parented by dominant and subdominant **a** adult males and **b** females. For each sampled year, the pack alpha and number of pups excluded as offspring of the alpha or subdominant adults are given

a *MALES*						
Pack	Year	Alpha	Pups excluded as offspring of Subdominants	Pups excluded as offspring of Alpha	Undetermined	Total pups sampled
Mbyamiti	91	mm2	3	–	–	3
Mbyamiti	92	mm2	6	–	1	7
Afsaal	91	am10	1	–	–	1
Afsaal	92	am10	1	–	–	1
Sand River	90	rm1	3	–	–	3
Doispane	90	unknown	–	1[a]	–	1
Doispane	93	sm10	7	–	–	7
Doispane	94	sm10	1	–	–	1
Skukuza	90	sm3	1	2[b]	–	3
Skukuza	91	sm3	2	–	–	2
Total pups			25	3	1	29
Total litters			9	2	1	10
b *FEMALES*						
Gomodwan	90	sf4	4	–	–	4
Gomodwan	91	sf4	1	–	–	1
Mbyamiti	91	df3	3	–	–	3
Mbyamiti	92[c]	df3	7	–	–	7
Afsaal	91[c]	af6	–	1[d]	–	1
Afsaal	92	af6	1	–	–	1
Sand River	90[c]	rf2	2	1[d]	–	3
Doispane	89	df1	1	–	–	1
Doispane	90	df1	3	–	–	3
Doispane	91	df1	2	–	–	2
Doispane	92[c]	df1	5	2[e]	–	7
Doispane	93	df1	7	–	–	7
Doispane	94	df1	1	–	–	1
Skukuza	89	sf1	5	–	–	5
Skukuza	90	sf1	3	–	–	3
Skukuza	91	sf1	2	–	–	2
Total pups			47	4	0	51
Total litters			15	3	0	16

[a] Minimum number not fathered by the alpha based on the presence of three unique paternal alleles
[b] Subdominant breeder was brother of alpha male
[c] Subdominant female bred
[d] Subdominant breeder was sister of alpha female
[e] Subdominant breeder was daughter of alpha female

The observed average PE(C) for 21 comparisons of known father/offspring combinations was 0.87. In the Doispane Pack, for year 1990, the dominant male was not identified. However, at locus 155, the mother's genotype was EF, and those of the three offspring were AE, EE, and CF, respectively. Consequently, there must have been two fathers. In the Skukuza Pack, in 1990, the alpha male (sm3) was directly excluded as father of two of the offspring whereas his brother, sm4, could not be excluded. Of the 29 pups sampled, alleles in 25 (86%) of the pups excluded all other adult males except the alpha.

For litters born in 1990 and 1991 in the Pretoriuskop pack and the 1992 Doispane pack, the alpha male could not be identified based on behavioral data so reproductive success could not be assigned. However, we were able to identify the father of two pups sampled in the 1990 Pretoriuskop pack as sm8 and the father of two pups sampled in 1991 as sm6, the brother of sm8. In the 1992 Doispane pack, five sampled pups were parented by the alpha female (df1) and a male (sm11) who disappeared early in the next breeding season. The two remaining pups from that year were the offspring of the subdominant female df8 who was the daughter of df1. The father of these two pups, sm10, was the likely brother of sm11 ($R = 0.80$). However, we could not determine which individual had alpha status in the pack for this breeding season. We have no behavioral information on sm11, and sm10 was observed to mount the alpha female (df1) twice in the 1992 breeding season. Presumably, sm 11 may have initially been the alpha male and sm 10 reproduced with a subdominant female. Thereafter, sm10 became the dominant male after which sm11 disappeared.

Based on the exclusion analysis of adult females, we determined that 4 out of 51 pups (8%) were not the offspring of the alpha female (Table 2b). The mother/

offspring PE(C) for all 14 loci combined was 0.997. The observed average PE(C) for 28 comparisons of known mother/offspring combinations was 0.87. Subdominant reproduction occurred in three packs. In the 1991 Afsaal pack, af6 was the alpha and her subdominant sibling and littermate, af5 ($R = 0.44$), was the mother of the only pup sampled. In the 1990 Sand River Pack, both the alpha female (rf2) and her sister (rf1) had pups that attained at least 1 year of age. Finally, in the 1992 Doispane pack, both the alpha female (df1) and her daughter (df8) had pups reach reproductive age and had different mates.

Estimation of reproduction by dominant and subdominant individuals revcals strikingly similar patterns for males and females. Alpha males and females attain about 96% of their total reproductive success each year through their own offspring whereas subdominants achieve only about 10% of their reproductive success directly with the balance derived through their relationship to breeders. No pack adult was found to be unrelated to all pups within its pack after 1 year of residence.

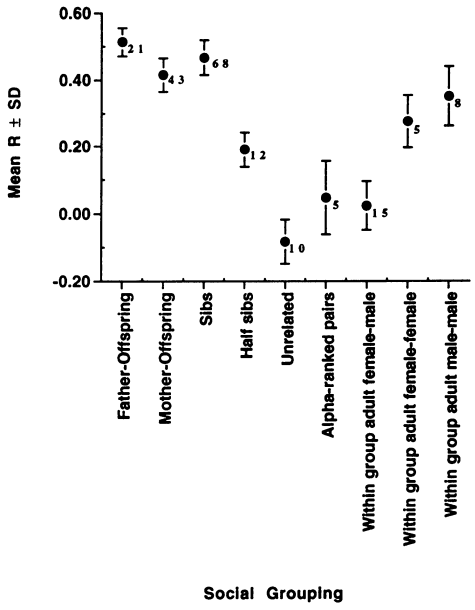

Fig. 3 Mean relatedness (R) for different relationship categories in wild dogs. The number of dyads examined for each category is indicated

Genetic relatedness

Several aspects of relatedness within and among packs could be discerned from relatedness (R) values estimated from the microsatellite data. Average estimated relatedness within packs was 0.265. This was significantly higher than the mean relatedness of packs generated by random assignment of individuals (mean $= -0.009$, range $= -0.021$ to 0.036, $P < 0.001$). In contrast, the average estimated relatedness for individuals from different packs, −0.03, was significantly lower than the mean relatedness value generated by random assignment of individuals (mean $= 0.003$, range $= -0.0002$ to 0.008, $P < 0.001$).

The alpha pair and adults of different sexes in each pack appear unrelated. The average relatedness of males and females that formed alpha pairs was 0.047 ± 0.108 ($n = 5$; range -0.267 to 0.170). Only one of five pairs is within a standard deviation of $r = 0.2$. The average within pack relatedness between adult males and females, excluding the mature offspring of the alpha pair was 0.023 ± 0.074 ($n = 15$; Fig. 3). These values are within one standard deviation of the observed mean relatedness of unrelated individuals and are close to the expected r value of zero for unrelated dyads. In contrast, the average within pack relatedness of adult females and that of adult males, 0.276 ± 0.080 ($n = 8$) and 0.351 ± 0.089 ($n = 5$), respectively, was significantly higher than that of unrelated individuals (permutation test, $P = 0.002$ and $P = 0.001$, respectively).

Dispersal

We rarely observed dispersal to other packs but a limited number of observations suggest dispersal may be motivated by a change in relatedness of alpha and subdominant adults. For example, in 1991, a change in the female hierarchy occurred in the Afsaal pack when a two year old female (af6) became the alpha female. The relatedness value between the long-standing subdominant female, af2, and the newly dominant, af6, was low ($r = 0.06$) indicating that they were related as half-sibs or were unrelated. Af2 left the Afsaal pack after af6 became dominant (Fig. 1) and dispersed 60 km to the Northeast to form the Thekwane pack. She then mated with an unknown male and had pups in 1992, three of which were sampled for this study. A comparison of relatedness values between af2 and two males (am2 and am4) who had emigrated to the neighboring Gomondwane pack shows high levels of relatedness (af2 and am2, $R = 0.49$; af2 and am4, $R = 0.55$; am2 and am4, $R = 0.49$). In addition, af2 had an average relatedness of $R = 0.22$ to the pups in the Gomondwane pack, suggesting she established a pack near her relatives. Interestingly, the dominant male of the Afsaal pack, am10, was not highly related to am2 ($R = 0.01$) or am4 ($R = -0.05$), both former members of the Afsaal pack who left in 1989 to form the Gomondwane pack.

The seven documented instances of interpack dispersal follow a pattern of dispersing to territories near close relatives and include three female and four male dispersal events involving up to three same-sexed individuals (af2; df2; df3; am2, am4; am8; sf4; sm5, sm6, sm8; and sm10, sm12). The movements of those individuals that were gentically sampled are depicted in Fig. 1. First, in all seven cases, the dispersing individuals were no longer related to one of the alpha individuals in their natal pack. Second, in six of the seven cases, the dispersing individuals settled in a territory that, at the

time of dispersal, was adjacent to the their natal pack. The six dispersal events to neighboring territories include: three females from the Doispane pack to the Mbyamiti pack; at least one female from the Skukuza pack to the Gomondwane pack, an adjoining territory at the time of dispersal; at least one female from the Afsaal pack to the Thekwane pack; three males from the Skukuza pack to the Doispane pack; four males from the Afsaal pack to the NewU pack; and four males from the Afsaal pack to the Gomondwane pack. In all seven dispersal events, dispersers settled in territories neighboring those that were occupied by close relatives as indicated by high R values. In each case, one of the dispersing individuals in each group attained an alpha rank.

If a pattern of dispersal to neighboring packs or areas near packs with relatives is a general one, then individuals from neighboring packs should have higher relatedness values than those found in a random distribution of individuals. Consequently, we examined the distribution of dyads having a Queller and Goodnight relatedness value of 0.4 or greater. This arbitrary bound is about 1 SE below the average relatedness value of known parent/offspring or sibling/sibling dyads (Fig. 3). Observed values of relatedness of female/female, male/male, and female/male dyads from neighboring packs were higher than those observed in at least 999 of 1000 random permutations of individuals (Fig. 4). In contrast, the observed mean relatedness among individuals from non-neighboring packs was lower than that in all 1000 random permutations. In addition, overall average relatedness values among individuals from neighboring packs were also significantly higher than those among individuals from non-neighboring packs for female/female comparisons ($R_{\text{neighbors}} = 0.0031$; $R_{\text{non-neighbors}} = -0.1117$; $P = 0.001$), male/male comparisons ($R_{\text{neighbors}} = 0.0272$; $R_{\text{non-neighbors}} = -0.0500$; $P = 0.001$), and female/male comparisons ($R_{\text{neighbors}} = 0.0029$; $R_{\text{non-neighbors}} = -0.0773$; $P = 0.001$).

Finally, ten individuals immigrated to our study area from the north in three separate events during the 5-year period of our study. Nine of the individuals were males, five of which were sampled for genetic analysis. Two immigrant males (mm1 and mm2) formed the Mbyamiti pack along with females df2, df3, and df4 from the Doispane pack. Five other immigrant males (am10, am11, am12, am13, and am14) joined the Afsaal pack. Two of these were sampled (am10 and am11) and are likely close relatives of mm1 and mm2 (range of R between the two groups; 0.20–0.59). Finally, an immigrant male (sum 3), who had migrated into the population with three other males, was sampled, but this group failed to establish a resident pack in the study area. The allele c at locus 342 was found in two of five of these sampled immigrant males and in their offspring and not in any other individuals in the population.

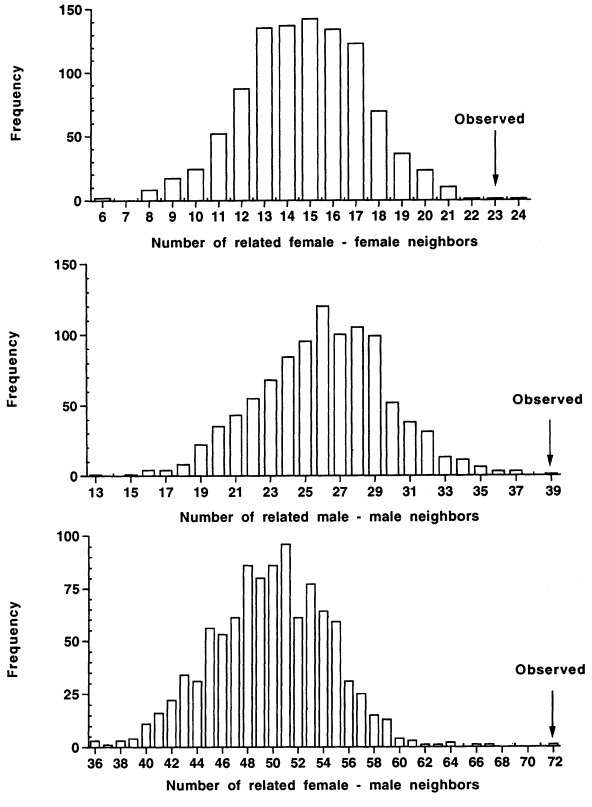

Fig. 4 Frequency distribution of highly related ($R > 0.4$) female/female, male/male, and female/male pairs produced by 1000 random samples of two individuals from the total population. The observed number of highly related individuals for same sex and different sex pairs that are neighbors are indicated in each plot

Genetic subdivision between packs

Differences in genetic subdivision were evident by sex. For females, a Northeast/Southwest division in the distribution of the two mtDNA genotypes (K1 and K2) was found. All females in the Pretoriouskop, Newu, Mbyamiti, Afsaal, and Thekwane packs had the K1 genotype whereas all the females in the Sand River, Doispane, Skukuza, and Gomondwane packs had the K2 mtDNA genotype. A similar comparison of male mtDNA genotypes revealed a mixed pattern. In six of eight packs where both males and females were sampled, K1 and K2 mtDNA genotypes were found in pack males.

We tested whether the mtDNA genotype pattern observed in males and females was significantly different form that generated by random permutation. In each of 1000 permutations, individuals were assigned randomly to packs of the same size as those actually observed. Our results indicated that the observed distribution of mtDNA genotype in females is highly significant as it occurred in none of 1000 simulations. In contrast, the mixed distribution pattern of male mtDNA genotypes occured in 13% of the simulations.

Finally, if the geographic segregation of K1 and K2 genotypes in females was a result of a matrilineal pattern of female dispersal then we would expect higher relatedness between females within each of the two genoytype subgroups. To test this prediction, we compared the

observed Queller and Goodnight relatedness values within each genotype subgroup with that between individuals randomly assigned to the packs in each of the two subgroups. We found that in 371 of 1000 simulations and 205 of 1000 simulations that male/male dyads and female/female dyads, respectively, had mean relatedness values that equalled or exceeded that observed within each of the mtDNA subgroups. In addition, a MDS plot showed no qualitative difference in the associations of packs with respect to the mtDNA subgroups (Fig. 5). These results suggest that the geographic division in female mtDNA genotypes does not indicate a geographic boundary between two female groups, each of very recent descent from different maternal ancestors. However, the Sand River pack was a consistent outlier in MDS plots of both male and female wild dogs.

Discussion

The social structure of wild dogs

Our microsatellite data confirm many aspects of the general model of African wild dog social structure. Packs in Kruger National Park are generally composed of an unrelated alpha male and female, and their adult siblings or close relatives. With few exceptions, pack pups are the offspring of the dominant pair. The average relatedness within packs was significantly higher than that found in random assignments of individuals to different packs. However, the high relatedness within each pack was structured according to sex. Although same-sexed founding adults were highly related, consistent with their being siblings, female/male dyads were not (Fig. 3). In a few instances, as exemplified by dm7 (Doispane pack) and am5 (Afsaal pack), a subdominant male was not a relative of the alpha male, rather he was a son of the alpha female and therefore was still related to her offspring. All ten of the subdominant males that were sampled appear to be related to pack offspring either through the alpha male alone, through the alpha female alone, or as a father. Thus, wild dog packs are extended family units consisting of parents, their offspring and their adult siblings or half-sibs.

Although previous studies suggested that the alpha pair parent the majority of pack offspring, the reproductive contribution of different members of a pack was always in question (Frame and Frame 1976; Frame et al. 1979; Malcolm and Marten 1982). Our results show that subdominants occasionally succeed in producing offspring that survive to one year of age and that subdominant reproduction occurred at similar levels in both males (10%) and females (8%). Multiple paternity occurred once in ten litters with an average of 2.9 pups sampled per litter (Table 2a). For one of the two cases in which the alpha male was excluded, a brother of the alpha was identified as the father of the offspring. In the other case, the subdominant breeder was not identified and may have not been included in our sample. Occasional extra-pair fertilizations by the alpha male's brothers may be tolerated because of indirect reproductive benefits and assistance the alpha male receives in provisioning and protecting his own offspring. Alternatively, an alpha male may simply be unable to detect and prevent the occurrence of extra-pair fertilizations and, unlike females, cannot discriminate between his offspring and those of subdominants.

It is well known that occasionally subdominant females have litters (Frame et al. 1979). In this study, we witnessed the occurrence of multiple mothers in four of nine packs and 4 of 16 pack years from which samples for genetic analysis were taken. In the one case of multiple maternity where fathers could be identified unequivocally, each female had a different mate. Overall, at 10 of 25 dens at which behavioral observations were made, more than one female produced pups (M. Mills, unpublished work). However, the microsatellite data suggest that subdominant females produced about 8% of the pups that survived to 1 year of age. Models of optimal reproductive skew suggest that an alpha female might be able to bias reproduction but that she may do so at a level that allows for some subdominant reproduction (Vehrencamp 1983).

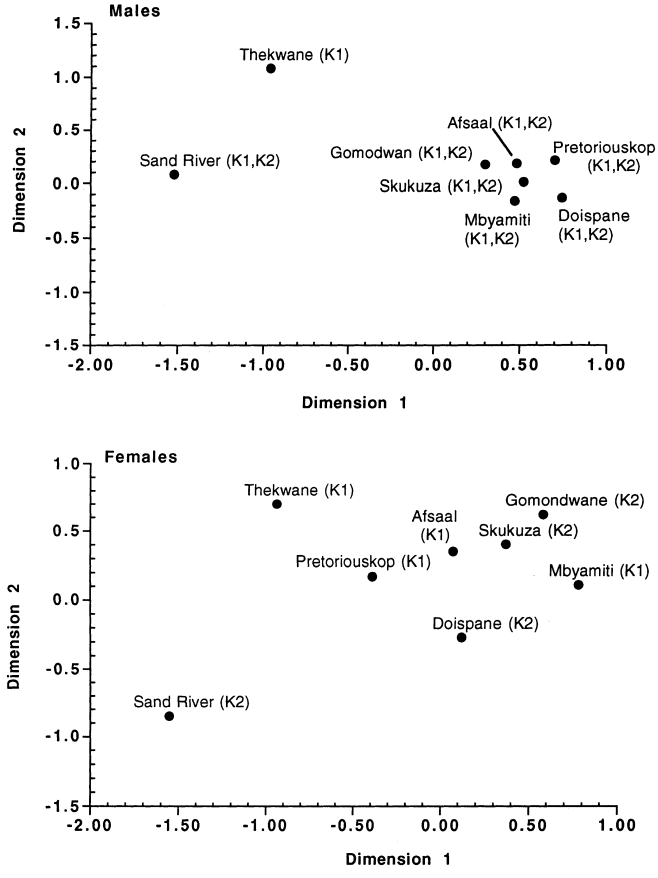

Fig. 5 Multi-dimensional scaling plot based on microsatellite allele frequencies of females and males in each pack if the pack is represented by two or more individuals. MtDNA genotypes present in each pack are also depicted

Behavioral data support the conclusion based on the microsatellite analysis that the offspring of subdominant females were less likely to survive to 1 year of age than the offspring of dominant females. Behavioral observations at six of ten dens where more than one female bred showed that all of the subdominant females' pups died before they reached one month of age (M. Mills, unpublished work). Although the cause of death of the subdominant female's pups was usually unknown, an alpha female was observed to kill some of the subdominant's pups. At one den, the alpha female's pups were seen to kill some of the subdominant's pups. This was an unusual case as the subdominant female produced her pups 3 months after the alpha female and consequently her pups were much smaller than those of the alpha female. These results are consistent with previous behavioral observations of severe competition between dominant and subdominant mothers and infrequent survival of subdominant pups (Frame et al. 1979).

Clearly, a unifying feature of wild dog packs is the high level of relatedness among individuals. We found no unrelated subdominant individuals persisting in wild dog packs of Kruger National Park, suggesting kinship with a dominant individual is a prerequisite for pack membership. Thus, communal rearing of pups by subdominant wild dogs is always directed towards closely related individuals (Malcolm and Marten 1982). Wild dogs differ from wolves, coyotes, and coatis (*Nasua narica*) which are known to give aid to unrelated young (Gompper and Wayne 1996; Malcolm and Marten 1982).

Dispersal patterns

Our analysis shows that male and female wild dogs are more closely related to individuals from neighboring packs than would be expected from a random distribution of individuals (Fig. 4). Therefore, both males and females may disperse within or near territories of their close relatives. For example, of the seven documented cases of dispersal within the study population (three female groups and four male groups), six were to areas adjoining their natal pack and the seventh was to a territory adjacent to close relatives (Fig. 1). However, dispersers that settle outside of our study are less likely to be detected, therefore our data are biased towards short distance dispersal. Nonetheless, dispersal within the study area is non-random and generally involves movements between adjacent territories (Fig. 1).

A pattern of dispersal favoring movements to neighboring packs may reduce the frequency and intensity of inter-pack encounters. In the gray wolf, interpack aggression is the largest source of mortality aside from that caused by humans (Mech 1987). In addition, dispersers may be more easily accepted into territories of neighboring packs that include some close relatives. In our study area, no mortalities were recorded due to interpack strife (M. Mills, unpublished work).

The reason that the Sand River pack is distinct in our MDS analysis is unclear (Fig. 5). The Sabie river is unlikely to act as a barrier to gene flow. For example, the long distance dispersers (e.g., mm1 and mm2) came from the northern portion of the Park, across the Sabie River. Moreover, males of the Sand River pack have high levels of relatedness to pups in the neighboring Thekwane pack suggesting that some migration has likely occurred across this boundary.

The timing of wild dog dispersal appears to be influenced by kinship. In all seven recorded dispersal events, dispersal was associated with the disappearance of a dominant and their replacement by a non-relative. In addition, dispersing individuals joined or established packs near their close relatives. For example, in the Afsaal pack, the subdominant af2 had a low level of relatedness to the new dominant female, af5 ($R = -0.06$). With the ascent of af5, af2 dispersed and established the Thekwane pack in a territory that was adjacent to that of her close relatives. The emigration of adults when they are no longer related to either of the alpha pair is consistent with inclusive fitness considerations. If either of the alpha individuals is replaced, some subdominants will be unrelated to future offspring and the relatedness of maturing pups to future offspring will decline from a sibling to half sibling relationship. In none of the packs did we discover adults that were unrelated to the alpha pair and yet persisted in a pack for more than a few months. Therefore, the advantages of sociality such as increased hunting success or mutual defence of food resources may not compensate for decreasing reproductive benefits caused by a change in the dominance hierarchy (e.g., Creel and Creel 1995; Gittleman 1989).

The composition of wild dog packs is consistent with their formation by the fusion of male and female sibling groups that are unrelated to each other. However, considering the frequency of dispersal to neighboring packs, these fusion events could conceivably involve distant relatives. A phenomenon in wild dogs that may help prevent the association of related dogs of different sexes is the occasional long-distance dispersal of males or the even rarer long-distance dispersal of females. Although we found little evidence that males disperse less frequently than females, other studies in the Masai Mara and the Serengeti Plains of Tanzania have reported differences in the dispersal distances of males and females (Fuller et al. 1992b; Frame et al. 1979). During our study, ten individuals were observed to have immigrated into our study population, nine of which were males. Eight of the males established packs and two (mm2 and am10) were verified to have reproduced. These individuals brought K2 genotypes to the Mbyamiti pack and the Afsaal pack and account for two out of the six mixed-genotype packs we sampled in Kruger Park (Fig. 1). Although not closely related, the sampled immigrants caused the introduction of a new allele (*c* at locus 342) into the study subpopulation. Because only a small number of immigrants per generation are necessary to overcome the loss of heterozygosity due to ge-

netic drift (Lacy 1987), these occasional long distance dispersal events may have a crucial role in maintaining the genetic viability of wild dog populations and reducing inbreeding. Long distance dispersal is important because there has been some concern in the past regarding the low level of genetic variability found in African wild dogs in Kruger National Park (Girman et al. 1993). However, it appears from the distribution of genetic relatedness among wild dogs of Kruger National Park that long-distance dispersal and the formation of packs by non-related sibling groups is sufficient to avoid inbreeding. In conclusion, despite the ubiquity of within pack kinship ties, the dispersal behavior of wild dogs limits opportunities for incestuous matings.

Females within a pack all shared the same mtDNA genotype whereas males often did not (Fig. 1). The differences in heterogeneity and geographic segregation of mtDNA genotypes may reflect the maternal inheritance of mitochondrial DNA combined with a pattern of local dispersal. All five of the sampled alpha pairs had low levels of relatedness, suggesting a system of dispersal and pack formation that precludes related individuals of different sexes from forming packs. Offspring will have the same mtDNA gentoype as their mother but this may differ from that of their father. Because the frequencies of the two mtDNA genotypes found in our study population are nearly equal, there is approximately a 50% chance that offspring will have a different mtDNA gentoype from their father.

Consequently, although local dispersal would be expected to maintain a pattern of mtDNA genotype similarity between neighboring packs for females, it may not for males. In contrast, microsatellite relatedness will decay rapidly overtime due to independent assortment of alleles in progeny (e.g., Packer et al. 1994) and consequently, geographic structure may be less apparent (Fig. 5).

In conclusion, we have demonstrated that African wild dog packs in Kruger National Park generally consist of an unrelated alpha male and female, their subdominant close relatives, and their offspring. The offspring that survive to 1 year of age are predominantly, but not exclusively, form the alpha pair. In addition, the patterns of relatedness seen in wild dogs in Kruger National Park, appear to influence both the timing of dispersal and the destination of dispersal. Individuals disperse when their relatedness to the alpha pair is low and then tend to establish territories near close relatives. Occasional long-distance dispersal may act to infuse new genetic diversity into the population. The combination of occasional long-distance migration and a pack structure that inhibits inbreeding maintains high levels of genetic variability in the African wild dogs of Kruger National Park.

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