Is incest common in gray wolf packs?

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Wolf packs generally consist of a breeding pair and their maturing offspring that help provision and protect pack young. Because the reproductive tenure in wolves is often short, reproductively mature offspring might replace their parents, resulting in sibling or parent–offspring matings. To determine the extent of incestuous pairings, we measured relatedness based on variability in 20 microsatellite loci of mated pairs, parent–offspring pairs, and siblings in two populations of gray wolves. Our 16 sampled mated pairs had values of relatedness not overlapping those of known parent–offspring or sibling dyads, which is consistent with their being unrelated or distantly related. These results suggest that full siblings or a parent and its offspring rarely mate and that incest avoidance is an important constraint on gray wolf behavioral ecology. *Key words: Canis lupus*, gray wolves, inbreeding, incest, microsatellites. *[Behav Ecol 8:384–391 (1997)]*

G ray wolves (*Canis lupus*) live in packs that generally contain a breeding pair and their offspring of one or more litters (Mech, 1970; Murie, 1944). Additionally, packs in the wild may include siblings or earlier offspring of one of the breeding pair (Mech and Nelson, 1990). Some packs may at least temporarily contain unrelated individuals (Mech, 1991; Meier et al., 1995; Messier, 1985; Peterson et al., 1984; Van Ballenberghe, 1983). Recently, a molecular genetic study showed that 8% and 44% of Minnesota and Denali wolf packs, respectively, included individuals unrelated to the breeding pair and their offspring (Lehman et al., 1992; Meier et al., 1995). However, the common elements of all long-established wolf packs are the breeding pair and their offspring.

The origin and genetic relationships of the breeding pair have been the subject of conjecture. Many pairs are formed from individuals that have dispersed from different packs, met, and pair-bonded (Rothman and Mech, 1979). Additional ways in which a breeding pair can develop include (1) an unattached lone wolf replaces one of the breeding pair that had dispersed or died (Fritts and Mech, 1981; Mech and Hertel, 1983); (2) an offspring replaces one of the parents (Mech, 1995; Mech and Hertel, 1983); (3) parents breed with offspring; and (4) siblings breed with each other. Incestuous matings between parent and offspring or among siblings have been recorded in captive wolves (Medjo and Mech, 1976; Packard et al., 1983) and on Isle Royale, Michigan, where wolves have no other choice than to mate with close relatives because of a lack of immigration from the mainland (Wayne et al., 1991).

Although there has been some speculation, the frequency of incestuous matings in the wild is unknown. Haber (1977: 246) believed that "there is a high degree of genetic isolation between unexploited wolf packs in the wild, that there is intense inbreeding and hence increased homozygosity within packs." Peterson et al. (1984), Shields (1983), and Theberge (1983) assumed that inbreeding was common in wolves, although they disagreed on its significance or the degree to

which it would be detrimental. Mech (1987) held that the high frequency of wolf dispersal would help ensure a high level of outbreeding in wolf packs, but that occasional dispersal to nearby packs would result in some cousin–cousin matings. In captive wolves, incestuous mating can lead to inbreeding depression (Laikre and Ryman, 1991), but it does not always (U. S. Fish and Wildlife Service, 1982). In the wild, inbreeding can persist for decades without population extinction, although some researchers believe that it may be the reason small populations do not increase in size (Peterson and Page, 1988; Wayne et al., 1991).

Nonreproductive, maturing wolves generally help provision and protect young (Haber, 1977; Mech, 1988; Murie, 1944), and as reproductive tenure in wolves is often short (Meier et al., 1995), helper wolves have a significant chance to reproduce, possibly within their natal pack. Therefore, because of the uncertainty about the origin of breeding wolf pairs and to better understand the role of inbreeding in wolf social behavior, we assessed the genetic relatedness of mated pairs in wolf populations whose mortality is minimally affected by humans. To do this, we used hypervariable simple repeat loci, or microsatellites (see reviews in Bruford and Wayne, 1993; Queller et al., 1993). Microsatellite loci have been used to assess paternity (e.g., Amos et al., 1995; Hagelberg et al., 1991; Morin et al., 1994; Schlotterer et al., 1992), to measure population differentiation (Paetkau and Strobeck, 1994; Roy et al., 1994), and to assess relatedness of individuals within social groups (Macdonald et al., 1994).

Because microsatellites are abundant in the mammalian genome, many loci can be surveyed and used to accurately measure relatedness (Chakraborty et al., 1988). In this study, we surveyed 20 microsatellite loci in two wolf populations and calculated relatedness between parents and offspring, among siblings and between mated pairs. We predicted that if avoidance of close inbreeding is an important constraint on wolf behavior, then incestuous matings should be uncommon and few mated pairs should be as closely related as parent and offspring or siblings.

Study areas

Denali National Park and Preserve ("Denali") is an area of 24,400 km² in central Alaska, USA. Elevation ranges from 150

to 6194 m, with a third of the area permanently glaciated. The remaining area ranges from subarctic tundra to coniferous and deciduous forest. The Denali wolf population increased during this study from four to eight wolves per 1000 km² in the parts of the park and preserve inhabited by wolves. Pack size ranged from 2 to 29, and mean territory size was about 1000 km² (Meier et al., 1995). Denali wolves preyed on moose (*Alces alces*), caribou (*Rangifer tarandus*), Dall's sheep (*Ovis dalli*), and beaver (*Castor canadensis*). Wolves were legally protected from killing by humans in 9200 km² of parkland, whereas limited killing was allowed in the 15,200 km² surrounding the protected area. Only eight wolves were known to have been killed by humans within this buffer area during the 8 years of this study.

The Superior National Forest (SNF) study area comprises 2060 km² in northeastern Minnesota, with elevations of 325–700 m. Vegetation is a mix of coniferous and deciduous forest and is inhabited by whitetail deer (*Odocoileus virginianus*), moose, and beaver, which are the primary prey of wolves. Wolf density has remained relatively stable at about 25 wolves per 1000 km² (Mech and Goyal, 1995). Pack sizes ranged from 2 to 15, and territory size ranged from 80 to 400 km² (Mech, 1986, unpublished data). Although wolves are legally protected in Minnesota, a few are still killed illegally each year.

METHODS

We sampled 130 wolves from 25 packs in Denali from 1986 to 1994 and 33 wolves from 6 packs in the SNF from 1988 to 1993. The sampled packs represent a small subset of the Greater Denali population that is a part of a largely continuous array of populations connecting the two sampling localities (Mech, 1970). In 10 Denali packs and all SNF packs, the mated or breeding pair was sampled. We anesthetized wolves by darting them from a helicopter (Denali) or by hypodermic injection when caught in traps (SNF). Wolves were fitted with radiocollars and ear tags, and 5–10 ml of blood was drawn by venipuncture into heparinized tubes. Wolves were located by aerial telemetry at approximately weekly intervals and observed (Mech, 1974). Most observations were made from October through March (SNF) or through May (Denali). Individual radio-collars functioned up to 4 years. We attempted to recapture wolves and replace expired radio-collars as many times as necessary to maintain continuous monitoring during the study.

We isolated white blood cells from whole blood in the laboratory and then froze them until needed. DNA was extracted from white cells by standard methods (Sambrook et al., 1989). The Denali and SNF populations had been previously analyzed for variability in 10 microsatellite loci and found to be similar in levels of heterozygosity, allelic diversity, and in the equability of allele frequencies (Roy et al., 1994). Consequently, estimates for various categories of relatedness should be similar in both populations.

In each population, we defined three social groupings based on behavioral criteria: mother–offspring, siblings, and mated pairs. A mated pair was defined as a radio-tagged male and female older than 2 years that traveled together for at least a few weeks. Most mated wolves were also breeding pairs as they remained alone together through the breeding season and produced pups. In larger packs, even when other adults were present in the pack, we identified mated pairs by behavioral attributes such as jointly leading the pack when traveling, close association with one another, and joint attendance at dens.

We defined individuals as mother and offspring if young were observed with the female of the mated pair defined above and if no other adult females were present in the pack.

We defined siblings as young born together in a pack with only a single known pair of mated adults. However, the apparent breeding female could conceivably have been a recent replacement of the actual mother of pack offspring, and the putative father could have been incorrectly assigned due to the possibility of extrapair copulations. Extrapair copulations have been documented with molecular genetic techniques in a wide variety of vertebrates, even in species thought to be monogamous based on behavioral observations (e.g., Burke and Bruford, 1987; Creel and Waser, 1994; Gottelli et al., 1994). Consequently, we determined if either of the mated pair could be excluded as a parent by documenting the presence of unique alleles in their putative offspring (Bruford et al., 1992). We calculated the exclusion probability per locus (PE_i) following Chakraborty et al. (1988):

$$PE_i = (1 - \delta - \beta)^2,$$

with δ and β being the allele frequencies found in an offspring. Combining the probabilities for all loci (Chakraborty et al., 1988) as follows:

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

yielded the proportion of randomly chosen adults in the population that could be expected to be genetically excluded as the father or mother of a given offspring.

Captive populations

To determine the correspondence of molecular genetic estimates of kinship with known relatedness, we obtained blood samples as above from two captive wolf populations with documented genealogies, the Julian pack and the Forest Lake colony. The Julian pack is located in Julian, California, USA, and was founded with two wild-caught individuals thought to be from different locations in central Alaska. We obtained samples from the single mated pair and their nine offspring of different years. The Forest Lake colony is located near Forest Lake, Minnesota, USA, and includes individuals from a large pedigree of wolves (Packard et al., 1983) with relationships ranging from inbred siblings to unrelated individuals. The 20 individuals we chose for analysis are a limited subset of the Forest Lake colony wolves, having relatedness (*r*) values ranging from 0 to 0.5 calculated from the pedigree (Falconer, 1983).
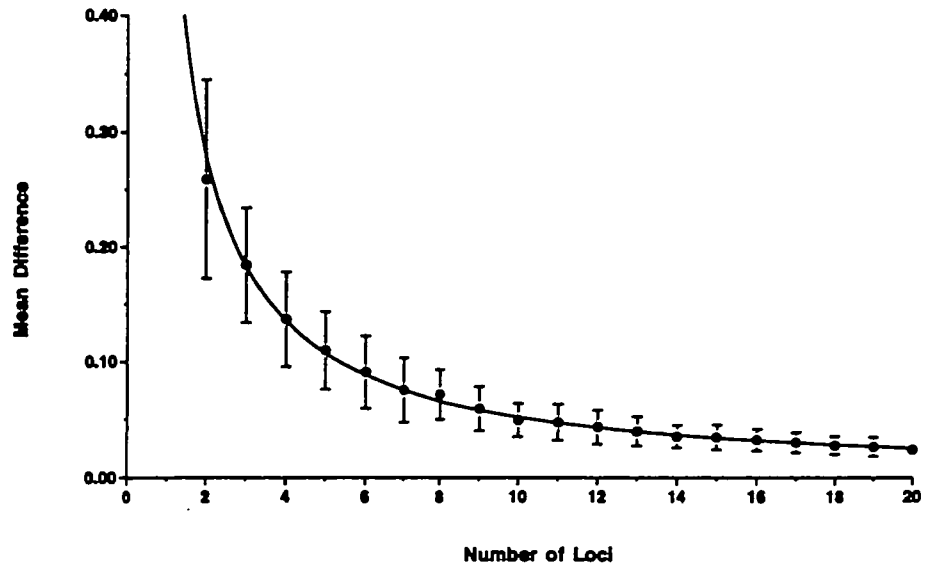
Microsatellite analysis

We used 20 $GT_{(n)}$ polymorphic microsatellite loci identified from a domestic-dog genomic library (Ostrander et al., 1993). Detection of microsatellite alleles from genomic DNA was achieved by end-labeling one primer by a standard $^{32}\gamma$-ATP (Amersham) and T_4 polynucleotide kinase reaction (Sambrook et al., 1989) and performing 28 cycles of polymerase chain reaction amplification in a 25-ml reaction volume using 50 ng of target DNA, 2 mM $MgCl_2$, and 0.8 U of Taq DNA polymerase (Promega). Reaction conditions were denaturation at 94°C for 45 s, annealing at 50°C or 55°C for 45 s, and extension at 72 °C for 60 s. We then mixed 3 μl of each product with 2 μl of formamide loading dye and heated it to 94°C for 5 min before being loaded onto a 6% sequencing gel containing 50% (w/v) urea. A M13 control region was run adjacent to the samples to provide an absolute-size marker for the microsatellite alleles. Gels were then autoradiographed overnight.

Statistical analysis

Because pedigree data were not known for wild-caught wolves, we used the Queller and Goodnight (1989) index of relat-

Figure 1
The decrease in the mean difference between consecutive relatedness estimates as a function of the number of microsatellite loci analyzed. The curve is described by the following equation: mean difference = 0.831 (number of loci)$^{-1.41}$, r = .998. Error bars indicate 1 SD above or below the mean value.

edness (R) to estimate kinship. This index weights each allele inversely by its frequency in the population, so that rare alleles are given a relatively higher weight. If a sample adequately represents a population in a Hardy–Weinberg equilibrium, the index values obtained for parent and offspring or for full siblings should approach 0.5. Overall, the index values vary between −1 and 1. The Queller and Goodnight index of relatedness was calculated for any two individuals (dyads) as follows:

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

The equation is summed over all loci and alleles. P^* is the population frequency of each allele excluding the compared individuals. Px and Py are the frequencies of each allele in the compared individuals, respectively (i.e., 0.5 or 1 depending on whether the individual is a heterozygote or homozygote). This index is not symmetrical, so reciprocal comparisons are not expected to equal each other (Py/Px). To accommodate for this discrepancy, we calculated the denominator values and numerator values for each combination (Py/Px and Px/Py), and summed them prior to the division. This procedure yields an average estimate of relatedness between the two individuals compared. Standard deviations for the relatedness values were estimated by jack-knifing over all loci (Queller and Goodnight, 1989).

Because of technical limitations, not all individuals could be typed for all 20 microsatellite loci. Consequently, we estimated the number of loci needed to estimate relatedness adequately by rarefaction analysis (e.g., Lehman and Wayne, 1991). We selected a locus at random, calculated the Queller and Goodnight relatedness value, and then selected another locus without replacement and recalculated the relatedness based on these two loci. The sampling was repeated without replacement until all 20 loci were selected. We then expressed the difference between consecutive samplings as a function of the total number of loci drawn. We repeated this procedure 100 times and calculated mean difference values (Figure 1). Descriptive statistics are given as mean values ±1 SD.

RESULTS

Rarefaction analysis showed that estimates of relatedness varied little after about 10 loci were sampled (Figure 1). For example, values differed on average by less than 4% if 10 rather than 11 loci were used to calculate R. Consequently, as few as 10 loci provide consistent measures of relatedness. Wolf dyads scored for this study averaged 16 loci out of a possible 20 compared. Only 4 of 500 dyads were compared at fewer than 10 loci.

To determine the correspondence of known and estimated relatedness, we first analyzed wolves of known genetic relationships from the two captive wolf populations. In the Julian population, all comparisons were between parents and offspring or between siblings (r = .5), except for the two breeding adults, which are presumably unrelated. In the Forest Lake colony, comparisons included parents and offspring and siblings (r = .5), first and second cousins (mean r = .21), and unrelated individuals (r = .0). The average Queller and Goodnight estimator, R, for each of these relatedness categories, .50±.09, .20±.27, and −.09±.09, respectively, are within about 1 SD of the corresponding actual mean r value (Figure 2). The mean values of unrelated (r = 0) and sibling or parent-offspring dyads (r = .5) are significantly different, as in none of 1000 random permutations did the difference in means equal or exceed the observed difference. The range of R values for parent–offspring or sibling dyads is limited; only 2 of 65 dyads have R values < .25 (Figure 3). However, the presence of a few unrelated dyads with large R values was unexpected and may reflect mistakes in the genealogy or in the labeling of DNA samples.

In the SNF population, we identified five mother–offspring dyads that fulfilled the specified behavioral and genetic criteria for a parent–offspring relationship. Similarly, we identified 10 sibling dyads as found in the same litters and with alleles that did not exclude either putative parent. All other sampled individuals were excluded as parents. The average exclusion probability in both the SNF and the Denali population was greater than .999 and hence the likelihood of drawing at random another individual from the entire population that was consistent as mother for a given offspring was less than 1 in 1000. Finally, in the SNF population, we identified six mated pairs based on behavioral data and the absence of excluding alleles (Table 1). In the Denali population, we identified 5 mother–offspring dyads, 1 sibling dyad, and 10 mated pairs using behavioral data and the presence of excluding alleles (Table 2).
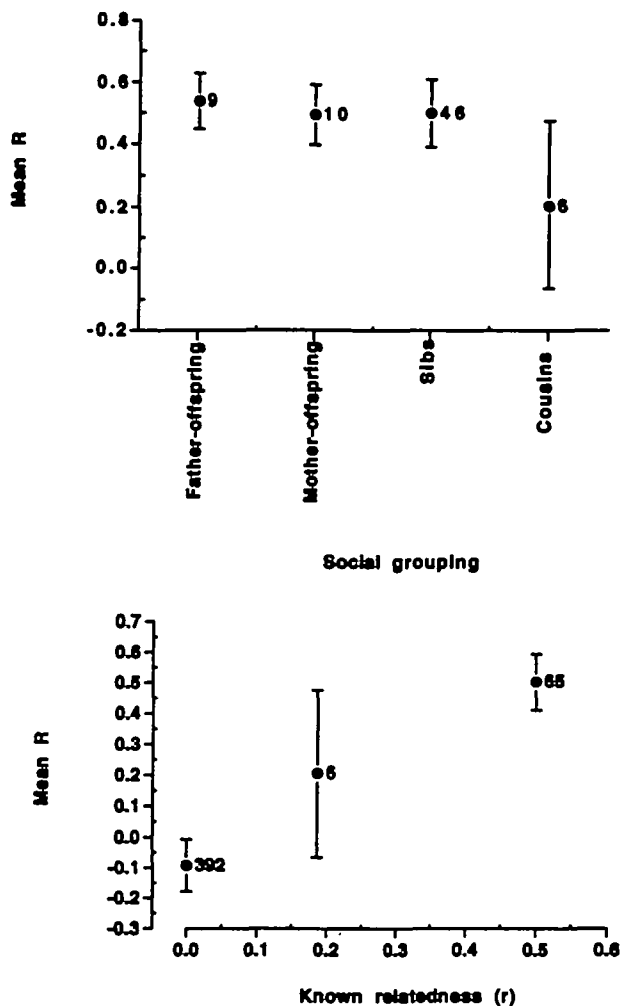
The Queller and Goodnight R values for the mother–off-

Figure 2
Mean relatedness (R) and SDs for different relationship categories in captive wolves. (Upper panel) R for mother–offspring, father–offspring, siblings, and first-and second-cousin dyads. (Lower panel) R for three categories of relatedness based on a known geneology. The number of dyads for each category is indicated next to the mean value. Error bars indicate 1 SD above or below the mean value.

spring and sibling dyads that we identified in wild wolves were close to the predicted value of $r = .5$ (Figure 4). Mated pairs had R values close to zero, the value expected for unrelated dyads. In the Denali population, the mean R of mother–offspring and sibling dyads was .57±.04 (range, .51–.63) and .54, respectively. These values were slightly higher than the corresponding values of .50±.10 (range, .40–.55) and .45±.08 (range, .22–.72) in the SNF population. To determine if R values for these related categories differed between the two populations, we randomly selected dyads from the pooled data to create samples of the same size as actually observed. The simulated populations had mother–offspring and sibling mean R values that differed by an amount equal to or greater than that observed in 117 and 366 of 1000 random permutations, respectively. Consequently, values of R are not significantly different in the two populations.

The mean R value of 6 mated pairs in SNF was −.054±.14 and of 10 Denali mated pairs was .05 ± .11 (Tables 1 and 2). These mean values are not significantly different because

mean values of R between mated pairs in simulated populations differed by a amount equal to or greater than that observed in 134 of 1000 random permutations. R values of mated pairs are within 1 SD of the observed value in unrelated, captive wolves and are more than 2 SDs below the mean for wolves related as mother–offspring or siblings (Figures 2 and 4). None of the R values for mated pairs in Denali or SNF overlaps those of mother–offspring or sibling dyads in either population. However, some alpha pairs may be slightly related considering the large variance in Queller and Goodnight relatedness values of captive wolves having known r values of .2 (Figure 2).

DISCUSSION

Because wolves live in packs that are primarily family units, there is considerable opportunity for incestuous matings and for reproductive succession by helpers. Most adolescent wolves disperse from their natal packs when ≤3 years old (Gese and Mech, 1991; Mech, 1987), but some remain longer or disperse only a short distance to nearby packs (Lehman et al., 1992; Mech, 1987; Meier et al., 1995). Consequently, incestuous matings are possible, especially with the death of one of the mated pair. Instead of dispersing, a young wolf could attempt to challenge a parent for breeding rights. In fact, in other carnivores, subdominants that are excluded by the dominant male from copulation or whose reproduction is hormonally suppressed may produce occasional offspring through sequestered matings or following the death of a dominant individual or a change in the dominance hierarchy (reviewed in Gompper and Wayne, 1996). A viable reproductive strategy in wolves might involve subdominant helpers forgoing dispersal for the possibility of direct reproduction within their natal pack ("biders"; Packard and Mech, 1980). However, observed incestuous matings in wolves occur primarily when wolves are prevented from outbreeding, such as in captivity or on Isle Royale (Medjo and Mech, 1976; Packard et al., 1983; Wayne et al., 1991). These observations suggest that wolves might breed incestuously only when dispersal opportunities are limited spatially.

We find no evidence in two natural wolf populations that mated pairs are related as parent and offspring or as siblings. None of the R values between members of 16 mated pairs overlapped those of sibling or mother–offspring dyads, and the mean value of relatedness, R, for mated wolves was > 2 SDs below the mean R of sibling and mother–offspring dyads (Figures 2–4). In fact, wolf 75 from SNF had three different mates during the period of the study; each time he paired with an unrelated individual rather than related packmates (Table 1). However, a larger sampling of mated pairs might reveal that some are highly related. The binomial probability that a sample of 16 mated pairs would yield no highly related pairs if their frequency in the population was 20% is only 0.03, but it is 0.19 if their frequency was only 10% in the population. Therefore, the formation of highly related pairs must be relatively rare, but we cannot exclude its possibility or the possibility of incestuous matings of more distantly related individuals.

These results imply an aversion to incestuous matings because wolves have far more opportunities to breed with a sibling or a parent than with an unrelated individual. Such opportunities include replacement of one of the breeding pair or the establishment of new packs by siblings. Breeding tenure is short; a preliminary estimate of mean tenure of breeding wolves in the Denali population is 4 years (Meier et al., 1995, in preparation). Our results suggest that adult offspring rarely replace a parent when the opposite-sex parent is present. Presumably, the negative fitness consequences of incestuous mat-
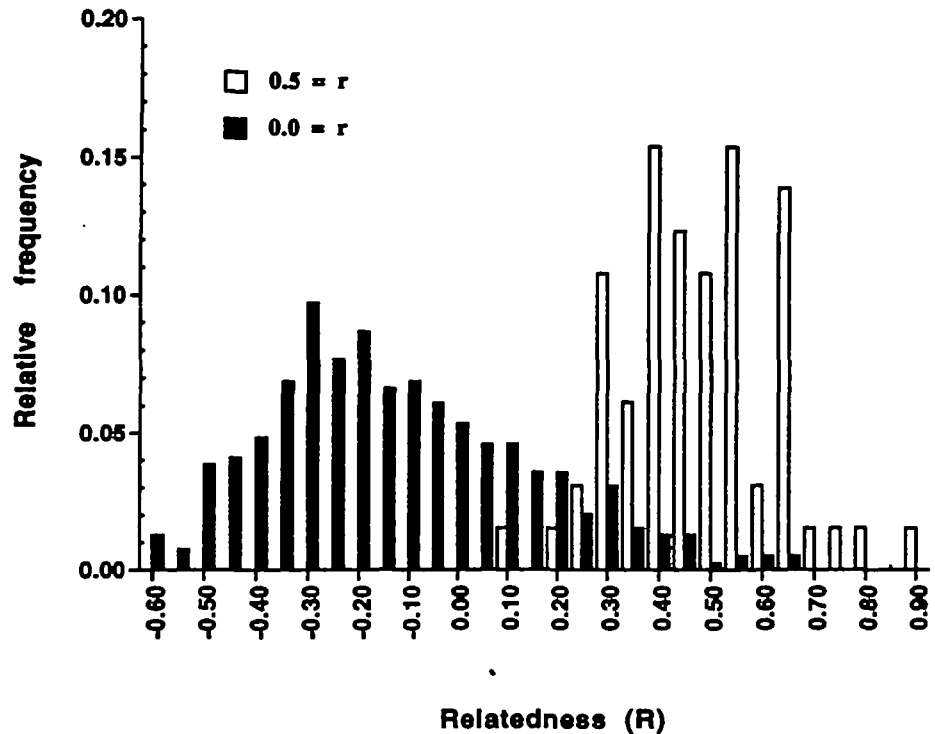
Figure 3
Frequency distribution of R
values for unrelated (r = .0, N
= 392) and highly related (r =
.5, N = 65) captive wolves.

Relatedness (R)

ings are not offset by the direct reproductive benefits of mating with a parent or sibling.

We cannot exclude other possible means by which inbred offspring may be produced in wolf packs. For example, inbreeding could result from multiple paternity due to the union of the breeding female and her mate and a son, from sequestered matings between parents and their offspring, or from matings between siblings. Such incestuous matings would be difficult to detect if they involved the female parent and her son. However, the insemination by one male of both his mate and daughters or matings between siblings would result in multiple litters. In the SNF, multiple litters were observed only rarely, and pack size is small (Mech, 1986), suggesting this is an uncommon source of inbred offspring. In Denali, packs are larger, and we have observed multiple litters in some packs that were of uncertain paternity (Meier et al., 1995), leaving open the possibility of father–daughter or sibling matings in large wolf packs. However, in a preliminary study, none of 10 adult mated dyads from large packs appears to be related as siblings or as parent–offspring (Meier et al., in preparation).

In sum, our results show that within wolf packs, mated wolves are rarely related as siblings or as parent–offspring. This observation suggests that in general, wolf packs are established by unrelated or more distantly related wolves. Offspring do not often, if ever, replace either parent unless the opposite-sex parent is first replaced by an unrelated wolf, nor do full siblings often become the breeding pair. Despite frequent opportunity, incestuous reproductive succession is not a common means to attain reproductive success.

Inbreeding avoidance may be one of the primary motivations for individuals to disperse (Pusey, 1987, 1996), although ecological and kinship factors critically influence the probability of dispersal (e.g., Creel and Waser, 1994; Koenig et al., 1992). In Minnesota gray wolves, interpack aggression is the largest source of mortality aside from that caused by humans (Mech, 1991). Consequently, the risks of dispersing and defending a new territory near hostile wolves might be sufficient cause for maturing wolves to remain in their natal pack where they have a chance to reproduce with a close relative. Over many generations, wolf packs would become inbred and the alpha pair would be genetically more similar than individuals

Table 1
Histories and relatedness of bonded wolf pairs in the Superior National Forest, Minnesota, USA

Male no.	Female no.	R [a]	Duration [b]	Pack	Pups?	Together (%) [c]	Fate
75	6755	.12	Aug '89–Nov '89	BL	No	11/13 (85)	6755 shot
75	257	.11	Oct '90–Mar '92	BL	Yes	69/100 (69)	75 left
75	313	−.23	Mar '92–May '92	LL	No	27/34 (79)	313 signal lost
93	313	−.08	Dec '91–Mar '92	KL	No	14/17 (82)	93 killed by wolf
253	273	−.17	Sep '90–Apr '95	PL	Yes	87/120 (73)	273 signal lost
453	451	−.08	July '93–July '94	FR	Yes	49/81 (60)	453 signal lost

[a] See Queller and Goodnight (1989).
[b] Period when wolves were together and radio-collared.
[c] Percentage of radio locations when pair was together.

Table 2
Histories and relatedness of bonded wolf pairs in Denali National Park, Alaska, USA

Male no.	Female no.	R[a]	Duration[b]	Pack	Pups?	Together (%)[c]	Fate
511	501	.12	Mar '93–Oct '94	SV	Yes	12/15 (80)	511 shot
223	227	.03	July '86–Jan '89	CW	Yes	137/187 (73)	223 killed by wolf
363	361	.19	Mar '89–Dec '89	CW	Yes	21/42 (50)	363 died, cause unknown
4520	529	.00	Mar '93–Oct '93	TU	No	15/27 (56)	529 killed by wolf
513	467	.12	Mar '93–Jan '95	EF	Yes	14/20 (70)	513 died, cause unknown
441	495	.19	Mar '92–Sep '92	FO	Yes	4/6 (67)	441 died, cause unknown
251	307	−.01	Feb '88–Nov '93	HQ	Yes	182/206 (88)	251 capture mortality
351	349	−.01	Oct '88–Feb '90	ST	Yes	59/64 (92)	Both killed by avalanche
515	499	−.24	Mar '93–Jan '94	TF	Yes	16/17 (94)	515 killed by avalanche
455	475	.12	Mar '92–present	ST	Yes	13/13 (100)	Active

[a] See Queller and Goodnight (1989).
[b] Period when wolves were together and radio-collared.
[c] Percentage of radio locations when pair was together.

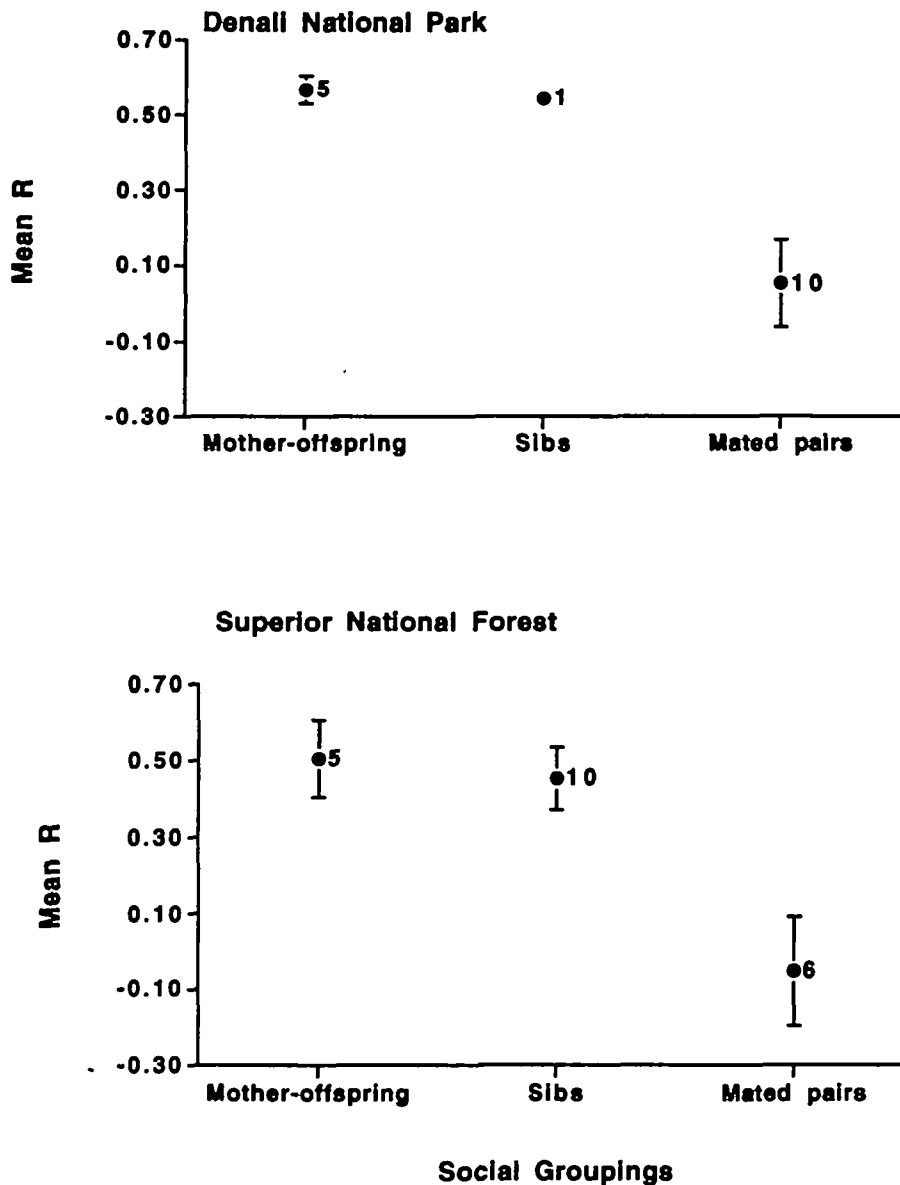Figure 4
Mean relatedness (R) and SDs for different relationship categories in wild wolves. The number of dyads examined for each category is indicated next to the mean value. Error bars indicate 1 SD above or below the mean value.

known to be unrelated. In naked mole rats, no immigration is tolerated into colonies, and they are entirely inbred (Reeve et al., 1990). The frequent pairing of unrelated wolves that we have observed ensures genetic heterogeneity within wolf packs and suggests inbreeding avoidance may be one of the primary reasons for dispersal from natal packs.

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