Original Article

Pack social dynamics and inbreeding avoidance in the cooperatively breeding red wolf

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For cooperatively breeding groups composed of close relatives, whether and how a group avoids inbreeding are questions of key evolutionary and conservation importance. A number of strategies for inbreeding avoidance may be employed by cooperative breeders, including extrapair reproduction, reproductive suppression, and juvenile dispersal. However, population-wide information on the prevalence of different strategies is difficult to obtain. We investigated the prevalence of inbreeding and potential mechanisms for inbreeding avoidance in a reintroduced population of the red wolf. Using long-term data on individuals of known pedigree, we determined that inbreeding among first-degree relatives was rare. Potential mechanisms for inbreeding avoidance included low levels of philopatric reproduction in spite of delayed dispersal, and reproductive suppression prior to dispersal. Inbreeding avoidance among siblings may have been further facilitated by independent dispersal trajectories, as many young wolves spent time alone or in small nonbreeding packs composed of unrelated individuals. The dominant pattern of breeding-pair formation involved the union of 2 unrelated individuals in a new home range. Replacement of 1 or both members of an existing breeding pair involved new immigrants to a pack or, in a small number of cases, ascendance of either resident offspring or adopted pack members to vacant breeding positions. Extrapair reproduction was rare, suggesting that it was not a major mechanism for outbreeding. We conclude that there are several prevalent behavioral strategies within the red wolf population that may work together to minimize inbreeding and any associated fitness costs, helping make cooperative breeding an evolutionarily viable strategy. *Key words: Canis rufus*, competition, lone wolves, monogamy, nonbreeding packs, pair formation. *[Behav Ecol]*

INTRODUCTION

The risk of inbreeding is a potential threat to the evolutionary stability of cooperative breeding when groups are composed of close relatives. As high levels of inbreeding have been associated with negative effects on fitness-related traits (reviewed in Pusey and Wolf 1996; Hedrick and Kalinowski 2000; Keller and Waller 2002), the study of mechanisms for inbreeding avoidance has been a topic of great interest to behavioral ecologists. Studies in diverse species have suggested that cooperative breeders can exhibit a range of behaviors that may help reduce inbreeding within groups, including extrapair reproduction, reproductive suppression of subordinates, and sex-biased dispersal (reviewed in Pusey and Wolf 1996; Packard 2003). Indeed, although isolated instances of inbreeding have been documented in many cooperative breeders, in most species the rate of inbreeding is thought to be quite low (Koenig and Haydock 2004).

Inbreeding rates and evidence for inbreeding avoidance have been studied most thoroughly in cooperatively breeding birds (reviewed in Koenig and Haydock 2004). Inbreeding avoidance has also been explored to some extent in cooperatively breeding mammals, but with the exception of a few well-studied species (e.g., meerkats: O'Riain et al. 2000; Griffin et al. 2003; Ethiopian wolf: Sillero-Zubiri et al. 1996,

Randall et al. 2007; African wild dog: Girman et al. 1997; Damaraland mole rat" Cooney and Bennett 2000; canid spp.: Geffen et al. 2011), most support for inbreeding avoidance tends to rely on anecdotal accounts. For many species, little is known regarding the prevalence of different strategies within a single population, and how they might work together to create a viable social system. In order to fully understand all possible social mechanisms through which harmful levels of inbreeding might be avoided, it is important to have a basic understanding of the different strategies that characterize the life cycles of individuals, and from this, discern the dominant strategy (or strategies) exhibited within a particular population. However, knowledge of key factors—such as when (or if) individuals disperse from their natal groups, how individuals spend their time before breeding, how breeding relationships are eventually formed, and by whom—is often difficult to come by. Such knowledge requires longitudinal, individual-based information on location and group affiliation, as well as detailed information on the sex, age, reproductive status, and relationships among individuals within a population (Pemberton 2008).

Cooperative breeding is widespread among canids (Moehlman 1986; Moehlman 1997; Mech et al. 1999; Packard 2003; Sparkman et al. 2011a), and potential mechanisms for inbreeding avoidance appear to vary among species. The Ethiopian wolf, *Canis simensis*, for instance, exhibits female-biased dispersal, thus limiting opportunities for mate formation between siblings (Sillero-Zubiri et al. 1996). Furthermore, although Ethiopian wolves tend to be socially monogamous, the frequency of extrapair reproduction appears to be relatively high, which may further reduce the risk of

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inbreeding when female offspring are philopatric and replace their mothers as the dominant breeder (Sillero-Zubiri et al. 1996; Randell et al. 2007). In contrast, the gray wolf, *Canis lupus*, tends to exhibit both social and genetic monogamy (reviewed in Packard 2003), with only rare instances of extrapair reproduction, suggesting that in this case reproductive suppression of subordinates and high rates of dispersal for both sexes may contribute more to low rates of inbreeding within packs (Smith et al. 1997; Vonholdt et al. 2008).

Inbreeding depression within captive populations of *Canis* species ranges from low to severe (Laikre and Ryman 1991; Ellegren 1999; Kalinowski et al. 1999; Fredrickson and Hedrick 2002; Lockyear et al. 2009; Rabon and Waddell 2010). In the wild, Scandinavian gray wolves recovering from a population bottleneck exhibit signs of severe inbreeding depression (Liberg et al. 2005). The reintroduced population of Yellowstone gray wolves, on the other hand, exhibits relatively low inbreeding coefficients (f), likely due to a relatively large founding population as well as mechanisms for inbreeding avoidance (Vonholdt et al. 2008). There is also evidence that inbreeding within packs in other gray wolf populations, as well as populations of other canid species, is rare (Smith et al. 1997; Geffen et al. 2011). Similarly, a wild population of Eastern wolves (*Canis lycaon*) appears to exhibit high levels of heterozygosity (Grewal et al. 2004; Rutledge et al. 2010). Nevertheless, there is still much to learn regarding inbreeding in wild populations, and the study of behavioral mechanisms for inbreeding avoidance has conservation as well as theoretical relevance, particularly as the risk of inbreeding is a major concern for small or declining populations and reintroduction projects often involve small numbers of founding individuals (Hedrick and Kalinowski 2000).

We investigated the prevalence of inbreeding among first-degree relatives (i.e., parent/offspring, siblings), and potential behavioral mechanisms for inbreeding avoidance in a reintroduced population of the cooperatively breeding red wolf, *Canis rufus*. The red wolf is an ideal system in which to investigate the fitness costs and benefits of cooperative living, as it constitutes a relatively closed, closely monitored population of known pedigree. Although derived from a captive population descending from only 14 founders, inbreeding coefficients of wild-born individuals are relatively low (mean $f = 0.10 \pm 0.05$, range 0–0.26) (William Waddell, Point Defiance Zoo and Aquarium, unpublished data). Furthermore, inbreeding events appear to be infrequent in the closely related Eastern wolf (Rutledge et al. 2010). Thus, we predicted that instances of breeding among first-degree relatives in the red wolf are rare and there are mechanisms for inbreeding avoidance active within this population.

Previous work has reported that red wolves primarily live in packs composed of a socially monogamous breeding pair and offspring of different ages (Phillips et al. 2003; Sparkman et al. 2011a). In spite of the fact that many young wolves will delay dispersal for up to 2 years, previous work has also demonstrated high rates of dispersal prior to reproduction, with only a few individuals remaining to breed in their natal pack (Sparkman et al. 2011b). Infrequent breeding in the natal pack could be sufficient for inbreeding avoidance, at least among parents and offspring. However, the extent to which inbreeding occurs has not yet been documented in the red wolf, and how dispersal and social behaviors both outside and within breeding packs might decrease the probability of inbreeding has not hitherto been explored.

To address these questions, we evaluated the prevalence of different strategies in the red wolf life cycle. Using long-term radio-telemetry data and a population pedigree, we explored prebreeding social behavior and its ramifications for the longevity of family bonds, and the prevalence of different mechanisms for breeding-pair formation, including natal philopatry, adoption of unrelated individuals into a breeding pack, and competition. We also assessed the frequency of extrapair reproduction as another potential mechanism for inbreeding avoidance. We predicted that strategies favoring the formation of breeding pairs between 2 unrelated individuals would be most prevalent. Note that we do not argue that any of these behaviors evolved exclusively as a result of direct selection for inbreeding avoidance; rather, our goal was to evaluate how various components of red wolf behavior might contribute toward that end (Moore and Ali 1984; Pusey and Wolf 1996).

METHODS

Monitoring methods and pedigree

Red wolves were reintroduced into the Alligator River National Wildlife Refuge in North Carolina in 1987, after extinction from their native distribution throughout the southeastern United States (McCarley and Carley 1979; U.S. Fish and Wildlife Service [USFWS] 1984). Between 1987 and 2007, 506 free-ranging wolves were captured primarily via foothold traps, equipped with very high frequency radio-collars and monitored to gather detailed information on location, pack affiliation, reproductive status, and timing of dispersal and death (Phillips et al. 2003). It is estimated that >95% of handled adult wolves were collared and that >90% of adults on the recovery area were "known" (A. Beyer, USFWS, unpublished data). Radio-collared wolves were monitored every 3–4 days from the ground or via fixed-wing aircraft. Wolves were aged by PIT tagging at den sites or during pup capture in early fall. Based on this intensive monitoring data, it has been reported that red wolves disperse between 1 and 2 years of age, and begin to breed between ages of 2 and 4 on average (Sparkman et al. 2011a, 2012).

The reconstruction of the pedigree for the red wolf population has been described in detail elsewhere (Adams 2006). Briefly, genetic material was obtained for 703 individuals and genotypes were collected at 18 microsatellite loci with an average heterozygosity of 0.65 (Adams 2006). To assign parentage, we used a maximum likelihood approach as implemented in the program CERVUS 2.0 (Marshall et al., 1998; Adams 2006) as well as field data on known pairings and spatial locations of individuals. When 1 parent was known we could successfully assign parentage 95% of the time at the 95% confidence level and 96% of the time at the 80% confidence level. When neither parent was known we could successfully assign parentage 88% of the time at the 95% confidence level and 99% of the time at the 80% confidence level using these 18 loci (Adams 2006). In total, we had genetic confirmation for the identity of both parents for 303 out of 408 individuals for whom parentage was inferred through field observations. One parent was known for an additional 101 individuals, and parentage was unknown for 194 individuals. From the resulting pedigree, we were able to identify breeding pairs and their corresponding offspring so as to be able to differentiate between what we define as related—that is, parent and offspring or siblings—and unrelated members of a pack—that is, adoptees/immigrants from other packs. Reintroduced red wolves do naturally hybridize with the coyote (*Canis latrans*), although management efforts selectively remove hybrid litters (Phillips et al. 2003). We were able to identify all instances of pair formation and hybridization between red wolves and coyotes (*C. latrans*). Using this information, we surveyed all instances of incestuous matings between first-degree relatives. All summary statistics and analyses were performed using JMP 8.0.2 (SAS Institute Inc.).

Prebreeding social behavior and inbreeding avoidance

We evaluated the prevalence of different social behaviors subsequent to dispersal from the natal pack, and their potential to contribute to inbreeding avoidance through dissolving social bonds between family members. Our long-term radio-telemetry records allowed us to identify 3 main social grouping behaviors exhibited by wild-born red wolves: solitary periods, membership in nonbreeding packs, and membership in breeding packs. Time periods were defined and quantified as the number of seasons in which a particular behavior was exhibited (i.e., Winter, Spring, Summer, and Fall). Solitary individuals that appeared to be traveling or residing in a particular home range for 1 or more seasons by themselves were designated as "lone wolves." Nonbreeding individuals that were found to be in the company of other nonbreeders in the same home range for 2 or more seasons were considered members of a nonbreeding pack. Individuals found for 2 or more seasons in a home range with 2 breeding individuals present were considered members of a breeding pack. Home ranges were determined from the 95% isopleths of utilization distributions, as estimated using kernel density estimators with fixed bandwidth estimated using the root-n bandwidth estimator (Steury et al. 2010; T. Steury, unpublished data).

We assessed the proportion of both sexes within the population that were lone wolves during at least 1 season, and classified them according to reproductive status during that time: prereproductive, postreproductive until death, ultimately nonreproductive until death, or between reproductive events. We also calculated the average age of lone wolves and length of time spent alone. Similarly, we also assessed the proportion of both sexes that were members of nonbreeding packs for at least 2 or more seasons and their reproductive status during that time. Since membership of nonbreeding packs fluctuated, with some wolves dispersing whereas others remained and new members arrived, we also compiled descriptive statistics on "subpacks," which we define as an aggregation of nonbreeding wolves that lasted 2 or more seasons longer than associations with other members (past or future) of a given pack. Note that in creating the category "subpack" we are not proposing a novel form of social organization per se, but simply devising an arbitrary but effective method to quantify the frequency and duration of different types of associations between individuals. We determined the composition of each subpack—numbers of each sex and presence of close relatives—the average age of members, and the average subpack lifespan (i.e., number of seasons spent together). Because the majority of nonbreeding subpacks were composed of male and female dyads that may have been attempting to form a breeding pair (see Results), we determined whether male–female dyads spent significantly longer together than other aggregations using Welch's test for unequal variance.

Breeding-pair formation and inbreeding avoidance

We identified breeding pairs as 2 individuals that were known to den and produce offspring. We identified and assessed the prevalence of 4 main patterns of breeding-pair formation: 1) the union of 2 lone individuals in a new home range, 2) the replacement of 1 lost breeder by a new breeder in an established home range, 3) the formation of a breeding pair composed of 2 new individuals in the absence of other individuals, or 4) the formation of a breeding pair composed of 2 new individuals in the presence of other unrelated individuals.

For patterns of breeding-pair formation (2)–(4), which involved the replacement of 1 or both breeders, we quantified the prevalence of the 4 different replacement mechanisms that were observed, the first of which could contribute to inbreeding, and remainder of which could contribute to inbreeding avoidance: replacement of a breeder by 1) resident offspring, 2) adopted immigrants, that is, individuals adopted by a pack with an intact breeding pair, 3) new immigrant individuals arriving after death or dispersal of a previous breeder, and 4) new immigrants that may have competed with and deposed a previous breeder. Concrete evidence for competitive breeder displacement is difficult to collect in wild wolves, although intraspecific conflict is known to occur and tends to be higher for breeding gray wolves than for nonbreeders (Mech and Boitani 2003). Thus, for (4), we considered the death or dispersal of a breeder after the arrival of a successor at some point after the previous breeding season as potential evidence for competition. Furthermore, because the majority of potential cases of competitive displacement involved males (see Results), we sought evidence that postbreeding male red wolves are more likely to be found outside of their breeding pack than females, with the prediction that females would be more likely to die in their breeding packs.

Extrapair reproduction and inbreeding avoidance

Using the population pedigree and information on pack composition, we were also able to quantify instances of multiple paternity (where pups from the same litter were fathered by 2 or more males) and instances of extrapair paternity (where a male fathered pups by 2 or more females), to determine whether these were common behaviors that could contribute to inbreeding avoidance within a cooperatively breeding family group.

RESULTS

Frequency of inbreeding

As predicted, breeding among first-degree relatives was rare. Breeding between parents and offspring occurred in only 4 out of 90 (4%) breeding pairs; similarly, breeding between full siblings also occurred in 4 (4%) breeding pairs. Two of four parent–offspring breeding events involved a single female, who bred with both her son, and then with their son. The remaining 2 parent–offspring breeding events involved father–daughter and mother–son pairings. One of four sibling breeding events involved siblings who bred together in their natal pack after their mother died during parturition, a second involved 2 siblings who dispersed from their natal pack together, and a third involved siblings born in consecutive years who independently dispersed to the area in which they bred. The fourth case involved a male who bred not only with his mother but also with his sister in the same year.

Prebreeding social behavior and inbreeding avoidance

The prevalence of lone wolf and nonbreeding pack behaviors subsequent to dispersal suggested that both may contribute to inbreeding avoidance. After dispersing from their natal packs, approximately 43% ($n = 386$) of monitored individuals spent 1 or more seasons as lone wolves (Figure 1). Of these, 28% ($n = 166$) were prereproductive and 72% were nonreproductive, that is, never became reproductive before death. The average age of pre- and nonreproductive lone wolves was 1.7 ± 0.9 years, and time spent as a lone wolf ranged from 1 to 13 seasons, with a mean of 3.0 ± 2.5 seasons. The sex of pre- and nonreproductive lone wolves was approximately evenly distributed (48% females and 52% males). Only 7% of lone wolves were postreproductive (i.e., did not reproduce again prior to death), and a mere 1% were between breeding
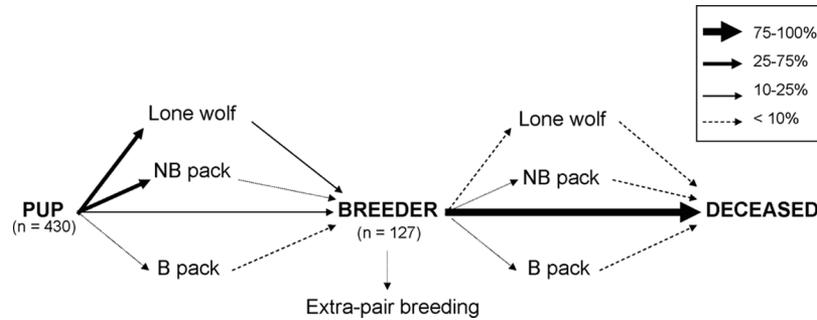
Figure 1
Prevalence of major social group behaviors employed by red wolves. Because only 30% of pups become reproductive, postbreeder percentages are based on the number of individuals that actually became reproductive. Note that all but 1 out of 21 instances of postbreeding extrapack behavior involved males. NB, nonbreeding, B, breeding (although not generally an individual's own breeding reproductive pack). Note that although not shown, some individuals may alternate between being alone, or in NB/B packs both before and after breeding.

events in different packs. Thus, in general, it appears that a large number of young wolves of both sexes spent time alone subsequent to dispersal from their natal packs, rather than in the company of siblings with whom they might otherwise have formed pair bonds (although this has only been documented in captivity, e.g., Packard et al. 1983).

After dispersing from their natal packs, 30% ($n = 386$) of individuals spent time associated with other wolves in nonbreeding packs, that is, packs in which there was no evidence of pups produced (Figure 1). Although nonbreeding packs were largely composed of pre- or ultimately nonreproductive individuals, approximately evenly distributed between the sexes (46% females and 54% males), 9 postreproductive individuals were also found within nonbreeding packs. There were 54 discrete nonbreeding packs with no temporal overlap among individuals within a given home range. The average size of nonbreeding packs was 2.4±0.8 wolves, with a range of 2–6. There were 91 subpacks within the 54 nonbreeding packs. The composition of the subpacks varied, but the majority were characterized by the presence of at least 1 male and 1 female (Table 1). Approximately 64% ($n = 91$) were male–female dyads. Male–female dyads spent significantly longer together than any other combination of nonbreeding individuals within a subpack, averaging 5.8±3.2 versus 3.2±1.2 seasons ($F_{1,66} = 27.8$; $P < 0.0001$). In every case, where there were originally more than 1 male and female in a nonbreeding pack, when only 2 individuals remained

in a pack it was a male–female pair. Nonbreeding packs were largely composed of individuals that were not closely related; there were only 3 cases where full siblings were found together in a nonbreeding pack. Only 12 individuals (6 males and 6 females) in nonbreeding packs went on to breed in the same home range. Thus, the majority of nonbreeding packs appeared to represent failed attempts at breeding-pair formation, and/or an aggregation of floaters available for breeding opportunities elsewhere. With respect to the former scenario, it is relevant to a goal of inbreeding avoidance that such a low number of subpacks contained first-degree relatives.

Formation of breeding pairs and inbreeding avoidance

There were 90 breeding pairs that were formed successfully during the study period, composed of 58 females and 69 males, as well as 3 cases where an entire family dispersed together to take up occupancy of a new home range. Seventeen percent ($n = 90$) of these breeding pairs involved coyotes; however, because the distribution of mechanisms for pair formation was similar for both red wolf–red wolf and red wolf coyote pairs (A. Sparkman, unpublished data), we retained these pairs in our analysis. There were 4 main mechanisms for formation of a new breeding pair: 1) joining of 2 lone individuals (54%), 2) replacement of 1 breeder either by an adopted immigrant or a son or daughter (24%), 3) replacement of both breeders by an adopted immigrant and/or a son or daughter (9%), or 4) the formation of a new breeding pair by new immigrants in the presence of one of the former breeders, or one or more other immigrants (12%) (Figure 2). The prevalence of strategy (1) is conducive to inbreeding avoidance, as it involves 2 individuals who have dissolved ties with closely related members of their natal pack. We describe the strategies involved in filling breeder vacancies in mechanisms (2)–(4), and their potential for causing or providing an alternative to inbreeding within a group, in more detail below.

Breeding of resident offspring
Inbreeding due to reproduction of resident offspring within their natal packs was rare. Only 8% ($n = 90$) of breeding-pair formations involved offspring—4 females and 3 males—that remained to breed in their natal packs. These events occurred through one or the other of two of the mechanisms listed above: when one (2) or both (3) parents were replaced as breeders. Two of the females in question bred during the spring after their mother's death, 1 with her father and 1 with a new pack member. The third female bred with a

Table 1

Number and percentage of the total number of subpacks for non-reproductive subpacks of various compositions

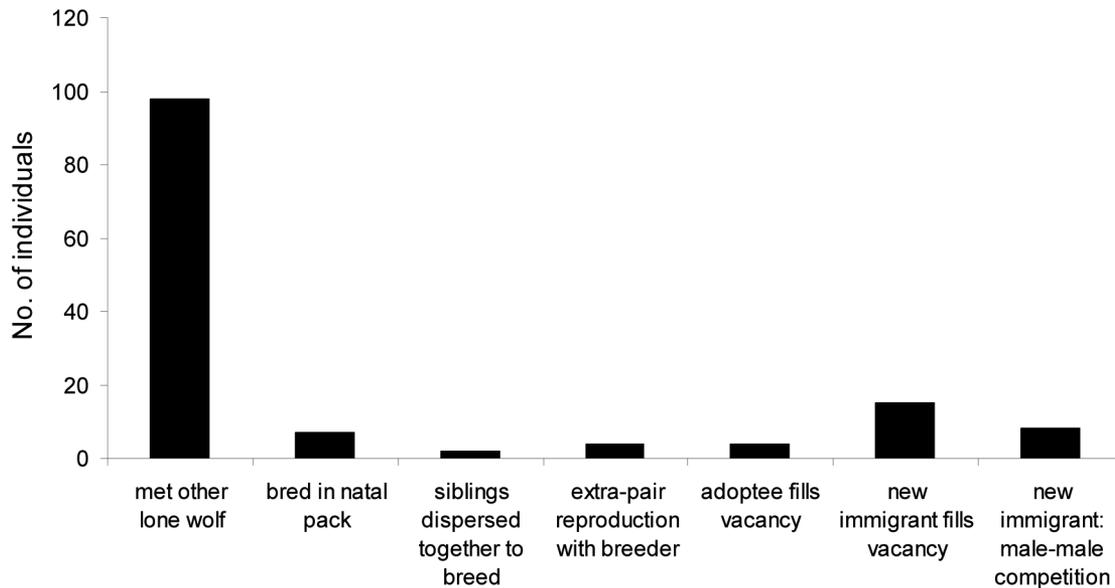| Composition	No. of subpacks	Percentage of total subpacks
5–6 Individuals		
MMMMFF	1	1.1
MMFFF	1	1.1
MMMFF	1	1.1
3–4 Individuals		
MMFF	2	2.2
MMMF	2	2.2
MMM	2	2.2
MFF	9	9.9
MMF	9	9.9
2 Individuals		
FF	1	1.1
MM	5	5.5
MF	58	63.7
Total	91	

Figure 2
Frequency of individual strategies for forming a pair bond. Note that some individuals fall into 2 categories (i.e., those that both bred in their natal pack and were involved in extrapair copulation), and some individuals formed more than 1 pair bond over their lifetime.

new pack member 2 years after her mother's death, and the fourth bred with her brother during the same year he bred with their mother. One male that bred in his birth pack bred with his mother after his father succumbed to an unknown fate; another male was his son, who bred with his mother/grandmother after his father dispersed, as well as with his sister (mentioned above). The third male bred with a new pack member the year after his mother died during parturition; his father remained in the pack during this time. Thus, breeding of resident offspring in their natal pack was rare, but in 4 out of 7 cases it involved inbreeding between first-degree relatives.

Breeding of adopted pack members
There were 43 instances where an unrelated individual was adopted by 35 of 90 breeding pairs. Of the 43 unrelated individuals that spent time in breeding packs, 3 were coyotes (2 male and 1 female) and 3 were wolves of unknown origin (1 male and 2 females). Of the 37 remaining individuals, there was a fairly even distribution between the sexes, with 15 females and 22 males accepted into breeding packs. The average age of adopted pack members was 2.4 ± 1.8 years, with a range of 0–8 years old. Two postreproductive males were accepted into breeding packs. Sixteen percent ($n = 43$) of adopted individuals later became reproductive themselves in other packs, and only 9% became reproductive in the pack in which they were an adopted member. Thus, adopted pack members were involved in mechanisms (2)–(4) for breeding-pair formation in only 4% ($n = 90$) of pairs. These individuals were involved in 1 out of 22 replacements of a single breeder (2), 1 out of 10 replacements of both breeders (3), and 2 out of 11 replacements of both breeders with other individuals present (4). These findings suggest that joining a breeding pack did not carry strong reproductive advantages either in the pack in question (actively displacing a current breeder or replacing a lost breeder), or elsewhere, and thus did not contribute substantially to inbreeding avoidance within the adopting pack.

Breeding of new immigrants
Breeding of new immigrants to a pack occurred at high enough frequencies to suggest that it may be an important

contributor to inbreeding avoidance, either through incidental arrival after a breeding position in a pack opened up or through male–male competition. Approximately 17% ($n = 90$) of breeding pairs were formed when a new immigrant arrived after the death or dispersal of a previous breeder and assumed a breeding position, either with the surviving mate or a new mate. There was also some evidence that an additional 12% ($n = 90$) of breeding-pair formations involved replacement of one member of a breeding pair (option (2) above) via male–male competition. Approximately half (55%) of transitions to a new breeder were potentially due to male–male competition, where the arrival of the new breeder or breeding of a subordinate was associated with the death/departure of the resident breeder between breeding seasons. Three of these cases involved sons displacing fathers (2 initially through extrapair copulation with their mother), 7 cases involved the arrival of a competitor and subsequent death/departure of the resident breeder within the same season, and 1 case involved the arrival of a competitor and subsequent dispersal of the resident breeder in the following season. There were 2 clear deaths due to intraspecific strife after the arrival of a competitor that resulted in 5- and 10-year-old males being replaced by incoming 2 year olds. In general, 3- to 10-year-old breeders were replaced by 1–3-year-old competitors, with the competitor always being younger than the resident breeder. We found little evidence of female–female competition, although 3 female breeder displacements could potentially have occurred by competition as defined above. One of these cases involved a female who took over from her mother after the death of her father.

Another line of evidence suggesting that male–male competition may have occurred more frequently than female–female competition lies in the higher frequency of males with postbreeding pack activity: 25% of male ($n = 69$) but only 2% of female ($n = 59$) breeders were located outside of their breeding pack after their last breeding event. Nineteen percent of male breeders spent time as lone wolves, 9% spent time in transient nonbreeding packs, and 3% spent time in stable breeding packs as nonbreeders (note that 4 out of 21 of males in this sample spent time both alone and with other wolves) ($n = 69$). Overall, males spent a mean of 7.3 ± 7.3

seasons (range 1–25) after dispersing from their breeding pack before death or censorship. The average age of postreproductive males outside of their breeding packs was 6.4±2.3 years (range 2–11 years). The only postreproductive female to leave her breeding pack and spend time elsewhere as a nonbreeder was 10 years old. She spent 1 season alone and 4 seasons with a postreproductive male (the first 2 seasons of which a nonreproductive female was also present) before being censored. Note, however, that approximately half of male dispersal events from their breeding packs appear to have been a consequence of mate loss, suggesting that there are reasons other than male–male competition that could create sex different frequencies in postbreeding pack lifespan.

Extrapair reproduction and inbreeding avoidance

Socially monogamous breeding pairs showed a high degree of genetic monogamy. From 1987 to 2007, within 174 litters produced by 90 breeding pairs (59 females and 69 males), there were only 4 instances of extrapair reproduction—2 involving multiple paternity, and 2 involving extrapair paternity. Two of the four instances involved mating between first-degree relatives described above (see Frequency of inbreeding). The third instance involved multiple paternity, where a female produced a litter both with her mate and with a lone male residing in a home range in close proximity. The fourth instance involved extrapair paternity by a male that produced a litter both with his long-term mate, as well as a litter with a female in an adjoining pack, whose long-term mate had previously been killed by a vehicle. This latter female went on to breed with a new resident male the following year, and the male continued to breed with his long-term mate. The low frequency of extrapair reproduction suggests that it was not a major mechanism for outbreeding within red wolf family groups.

DISCUSSION

Using long-term, population-wide data on the reintroduced red wolf, we explored the major social behaviors employed by red wolves throughout their lifespans (Figure 1). Consistent with studies in other cooperatively breeding species, including gray and Eastern wolves (e.g., reviewed in Koenig and Haydock 2004; Smith et al. 1997; Vonholdt et al. 2008; Rutledge et al. 2010; Steinglein et al. forthcoming), we found that in spite of prolonged associations among close relatives due to delayed dispersal, breeding pairs in the red wolf population were almost entirely composed of 2 unrelated individuals. We report a variety of behaviors, including independent dispersal trajectories, membership in nonbreeding packs of unrelated individuals, and a high prevalence of breeding-pair formation between unrelated mates, that may serve as mechanisms contributing to inbreeding avoidance.

Prebreeding social behavior and inbreeding avoidance

We observed few cases (4% of mated pairs) of siblings breeding together, thus although siblings may interact in the natal pack when delaying dispersal, they are unlikely to breed together. Almost half (43%) of the wolves in the study population spent time as lone wolves following dispersal from their natal pack (Figure 1). For these individuals, social bonds with siblings were effectively broken, increasing the likelihood that siblings will find unrelated mates. Furthermore, up to 30% of young wolves joined aggregations of other nonbreeding individuals (Figure 1), and only 3% of siblings were found in the same nonbreeding subpacks. This is critical given the

high frequency of male–female dyads among nonbreeding subpacks (Table 1), and that these dyads stayed together longer than other combinations of nonbreeders. These are indicators that such aggregations were incipient breeding packs which, if successful, could have increased levels of inbreeding if were largely composed of relatives. Thus, in general, the activities of dispersing young wolves favored mixing with wolves from other packs, rather than maintaining close ties with siblings. Interestingly, a recent study that compared rates of pairing with kin versus nonkin in several canid species suggested that selection for inbreeding avoidance via kin recognition mechanisms may be weak in canids, due to low rates of encounter with close relatives outside the natal pack (Geffen et al. 2011). Thus, our reported low rates of new pairs between close relatives may be attributable to low encounter rate rather than inbreeding avoidance per se.

Similarly, breeding between parents and offspring was rare (4% of mated pairs), in spite of the likelihood that some offspring that delayed dispersal to 1 or more years of age were physiologically capable of reproduction (Rabon 2009; Sparkman et al. 2011a). Two factors likely contributed to the low frequency of parent–offspring pairs. First, behavioral or physiological reproductive suppression of subordinates is widespread among canids and other cooperatively breeding mammals (e.g., reviewed in Solomon and French 1997; O'Riain et al. 2000; Packard 2003); second, the high rate of dispersal from the natal pack (Sparkman et al. 2011b), suggests that young wolves rarely compete with a same-sex parent for a breeding position (or at least succeed in doing so), and do not wait indefinitely for a position to become available. Although there were 7 instances of territory inheritance by resident offspring (Figure 2), a phenomenon previously reported in both gray and Eastern wolves (e.g., Mech and Boitani 2003; Jędrzejewski et al. 2005; Rutledge et al. 2010), most dispersed elsewhere to breed, which is consistent with other cooperatively breeding species (Dickinson and Hatchwell 2004; Russell 2003).

Although the inbreeding among first-order relatives is rare in the red wolf population, we have not demonstrated that red wolves are statistically less likely to mate with close relatives. Unfortunately, demonstrating this requires more than simply testing for inbreeding avoidance against a null model of random mating that incorporates spatial, temporal, and developmental constraints on mate formation. Ideally, an appropriate null model for a cooperative breeder should also incorporate the potential for a nonrandom preference for family members because, in the absence of behavioral mechanisms for inbreeding avoidance, these individuals may be the most easily accessible and energetically inexpensive mates, especially considering high costs of dispersal (e.g., Sparkman et al. 2011b). Indeed, it is the prolonged association between relatives of or near breeding age that raises the theoretical enigma of how such social systems avoid dangerously high rates of inbreeding in the first place. An additional challenge to modeling potential versus actual breeding pairs in our population of red wolves is the unknown availability of coyotes as potential mates. Nevertheless, we hope that future work will use the information we present here as a starting point for exploring a variety of alternate models that formally test for evidence of inbreeding avoidance among individuals of varying degrees of relatedness (e.g., see Geffen et al. 2011).

Breeding-pair formation and inbreeding avoidance

Patterns of breeding-pair formation in the red wolf favored the breeding of unrelated individuals. There were 4 major patterns of pair formation, the most prevalent being the pairing of 2 unrelated individuals in an otherwise unoccupied

home range (54%). This is not surprising given a previous finding that in approximately half of the cases where 1 member of a breeding pair is lost, the breeding pack is disbanded (Sparkman et al. forthcoming), necessitating that the majority of new pairs be formed independently. Furthermore, the population was expanding during the first few years of the study period (USFWS 2007), and many home ranges remained unoccupied during that time. Pairing of 2 lone individuals in this way is highly conducive to inbreeding avoidance, and is likely facilitated by high dispersal rates, a high proportion of individuals spending time as lone wolves, and the high frequency of unrelated male–female dyads even among pre- or nonreproductive wolves (Figure 1, Table 1).

Transitions resulting in the replacement of 1 or both members of a breeding pair occurred in the remaining 45% of cases (Figure 2). Interestingly, only 8% of total pair formations involved resident offspring replacing a parent as a breeder, and 4 out of these 7 cases involved inbreeding between parents and offspring or siblings. Thus, breeding in the natal pack, when it occurs, is often associated with inbreeding, suggesting that the low frequency of this strategy is in general an important factor in inbreeding avoidance.

Among cooperatively breeding species, a major mechanism for outbreeding can be adoption of unrelated immigrant individuals into a social group (e.g., Rood 1990). These individuals may take part in the regular activities of the pack, even providing care for young, with the possibility that, when the opportunity arises, they may eventually assume breeding dominance. Acceptance of "adoptees" into packs has previously been reported in gray and Eastern wolves (reviewed in Mech and Boitani 2003; Grewal et al. 2004; Jędrzejewski et al. 2005; Rutledge et al. 2010). Nevertheless, in our study, although unrelated individuals were occasionally adopted into a breeding pack, only 9% of red wolves employed this strategy (Figure 1), and breeding opportunities for these individuals was even rarer than for offspring within their natal pack (4% vs. 8%, respectively) (Figure 2).

It is worth noting that in our study, males and females were equally likely to be adopted, and 3 females and 1 male remained to breed in their adopted packs. This is surprising given the preponderance of male adoptees in gray wolves (Mech and Boitani 2003), and the lack of female immigrants observed in Yellowstone wolves (Vonholdt et al. 2008). Future studies should evaluate if differences in pack social structure in *Canis* species are attributable to species-specific factors, or whether strategies vary from population to population.

Another mechanism for outbreeding in wolves is through the arrival of new immigrants. Second to breeding-pair formation by 2 unrelated individuals on an unoccupied home range (54% of breeding pairs), immigration of new individuals into a pack to assume already-vacant breeding positions (17% of breeding pairs) was the most prevalent mechanism conducive to inbreeding avoidance in red wolves (Figure 2). There was also evidence that an additional 12% of breeding pairs were formed via male–male competition. Although there exists evidence of competition within groups for breeding status among cooperative breeders (e.g., Mumme et al. 1983; Reyer 1986), little is known regarding active displacement of resident breeders by competitors (but see Doolan and Macdonald 1996). Among gray wolves, intraspecific competition has been observed, but the extent to which this acts as a mechanism for breeder transition in a population is unknown (reviewed in Mech and Boitani 2003). In this study, there were 3 instances where a son took over from his father, although an additional 8 instances involved the arrival of an apparent competitor, followed by the death or departure of the breeding male (Figure 2). Evidence for female–female competition was negligible, and the fact that males were also much more likely to be found outside their breeding pack after vacating a breeding position suggests that competition for breeding positions, should it occur, is generally among males.

Interestingly, although more anecdotal accounts have suggested that the dominant pattern of pair formation in gray wolves corresponds to our option "1,", where 2 lone individuals form a pair bond (reviewed in Vonholdt et al. 2008), a recent study of the reintroduced Yellowstone population suggested that only 7% (2 out of 29) of pairs conformed to this pattern (Vonholdt et al. 2008). The remaining pairs were formed when packs split, a vacancy was filled by an unrelated individual, or a group of individuals dispersed to be joined by an opposite-sex group. It remains to be seen which pattern of pair formation is most prevalent in other wolf populations. It is possible that in the red wolf population, high levels of anthropogenic mortality exerting an additive effect on rates of pair-bond dissolution may be at least partially responsible for the high rate of pair formation by 2 lone individuals (Sparkman et al. 2011c). However, in spite of this disparity in the prevalence of different strategies for breeding-pair formation, both Yellowstone gray wolves and red wolves showed equally low levels of breeding between closely related individuals (Vonholdt et al. 2008, this study), suggesting that at any frequency, the employment of any or all of these strategies may culminate in similar levels of inbreeding avoidance.

Extrapair reproduction and inbreeding avoidance

Like the gray wolf (e.g., Smith et al. 1997; Vonholdt et al. 2008), the red wolf appears to be a rare case of both social and genetic monogamy. In 174 breeding events, there were only 4 exceptions, 2 involving resident offspring, and 2 involving individuals from adjoining home ranges. Furthermore, although there can be intraspecific variation in mating system in response to differences in ecological variables (Sun 2003), it is notable that although the red wolf population density rose steadily over the study period, reaching high and stable numbers from 2000 to 2007 (USFWS 2007), extrapair reproduction occurred so rarely so as to make any potential density-dependent increase in its frequency indiscernible. This suggests that although delayed dispersal of offspring and adoption of unrelated individuals into a pack may provide ample opportunity for extrapair reproduction, red wolves have a strong tendency to exhibit reproductive suppression of subordinates, thereby favoring the maintenance of monogamous pair bonds. Furthermore, high levels of territoriality may reduce the possibility of extrapack breeding with neighboring wolves. Thus, although extrapair reproduction may be an important mechanism for outbreeding in other cooperative breeders (e.g., Sillero-Zubiri et al. 1996; Randall et al. 2007; Young et al. 2007), red wolves appear to rely more on mechanisms compatible with a genetically monogamous mating system.

CONCLUSION

We found few instances of breeding between first-degree relatives in the reintroduced red wolf population, suggesting that there are elements to the red wolf life cycle that reduce the risk of inbreeding incurred by delayed dispersal of offspring. High dispersal rates, potentially accompanied by behavioral reproductive suppression prior to dispersal, likely contributed to low rates of inbreeding within a pack. Furthermore, the high proportion of young wolves spending time alone, or as members of nonbreeding packs primarily composed of unrelated individuals, could act as a barrier to breeding among siblings after dispersal. Outbreeding was at least partially facilitated by unrelated

individuals immigrating into a pack to replace 1 or both breeders (either serendipitously or actively via competition); however, although adoption of unrelated wolves into breeding packs did occur, these individuals seldom attained dominance. Similarly, red wolves did not appear to rely on extrapair reproduction with either adopted pack members or extrapack individuals for inbreeding avoidance, as they primarily exhibited both social and genetic monogamy, and 2 of the 4 instances of extrapair reproduction involved family members. Instead, the most significant guarantor of outbreeding appeared to be the high proportion of breeding pairs formed in new territories by 2 unrelated individuals.

In general, we conclude that an array of dispersal, postdispersal, and pair formation behaviors have the potential to work together to reduce rates of inbreeding, and any associated fitness costs, in the cooperatively breeding red wolf.

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