

# Sex-biased natal dispersal and inbreeding avoidance in American black bears as revealed by spatial genetic analyses

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## Abstract

We tested the hypothesis that sex-biased natal dispersal reduces close inbreeding in American black bears, a solitary species that exhibits nearly complete male dispersal and female philopatry. Using microsatellite DNA and spatial data from reproductively mature bears ( $\geq 4$  years old), we examined the spatial genetic structure of two distinct populations in New Mexico from 1993 to 2000. As predicted, relatedness ( $r$ ) and the frequency of close relationships (parent–offspring or full siblings) decreased with distance among female dyads, but little change was observed among male or opposite-sex dyads. Neighbouring females were more closely related than neighbouring males. The potential for inbreeding was low. Most opposite-sex pairs that lived sufficiently close to facilitate mating were unrelated, and few were close relatives. We found no evidence that bears actively avoided inbreeding in their selection of mates from this nearby pool, as mean  $r$  and relationship frequencies did not differ between potential and actual mating pairs (determined by parentage analysis). These basic patterns were apparent in both study areas despite a nearly two-fold difference in density. However, the sex bias in dispersal was less pronounced in the lower-density area, based on proportions of bears with male and female relatives residing nearby. This result suggests that male bears may respond to reduced competition by decreasing their rate or distance of dispersal. Evidence supports the hypothesis that inbreeding avoidance is achieved by means of male-biased dispersal but also indicates that competition (for mates or resources) modifies dispersal patterns.

*Keywords:* black bear, density, dispersal, DNA, inbreeding, kin recognition, *Ursus americanus*

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## Introduction

Patterns in natal dispersal and philopatry affect the demography and genetic structure of populations as well as the evolution of social behaviour (Waser & Jones 1983). In many species, one sex is typically philopatric while the other is prone to dispersal. Male-biased dispersal and female-biased philopatry are common characteristics of mammals, while the opposite is typical of birds (Greenwood 1980). Logically, sex-biased dispersal is expected to generate a nonrandom pattern of relatedness among adult cohorts residing in close proximity. Avoidance of inbreeding is one

among several hypotheses put forth to explain sex-biased dispersal. Greenwood (1980) proposed that the direction of the sex bias is a consequence of the mating system and competition for resources. In mammals, selection would favour philopatry in females, because they bear the burden of raising offspring (Greenwood 1980). Building on this connection between breeding system and dispersal, Wolff (1993, 1994) further developed the inbreeding avoidance hypothesis for mammals. He contended that daughters typically grow up in the absence of their father due to polygyny and the short breeding tenure characteristic of most male mammals. Thus, females would not have to disperse to avoid inbreeding. As a consequence of female philopatry, most males would be compelled to disperse to avoid inbreeding with their mothers or other female kin. As originally formulated, this argument does not explicitly

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address the point that costs of inbreeding accrue not only to the male, but also to the female relative with whom he mates. Nonetheless, in experimental and observational studies, inbreeding avoidance has been found to be a proximate, and some argue, an ultimate cause of sex-biased dispersal in mammals (Dobson 1979; Cockburn *et al.* 1985; Keane 1990; Wolff 1992; Loison *et al.* 1999).

We investigated the genetic structure of two American black bear (*Ursus americanus*) populations in New Mexico from 1993 to 2000 to examine the possible relationship between dispersal and inbreeding avoidance. Black bears are solitary omnivores, exhibiting overlapping home ranges (Amstrup & Beecham 1976; Garshelis & Pelton 1981; Horner & Powell 1990) and a promiscuous mating system (Schenk & Kovacs 1995; Kovach & Powell 2003; Onorato *et al.* 2004). A pronounced sex bias in dispersal has been observed (Rogers 1987a; Elowe & Dodge 1989; Beck 1991; Schwartz & Franzmann 1992), although observations have been limited in some potentially important ways. First, attempts to document black bear dispersal have been rare, probably owing to reluctance to radio-mark juvenile males due to the difficulty and expense of maintaining contact during a period of wide-ranging movements, as well as the potential for collar injuries resulting from rapid growth. Second, most studies of black bear dispersal have successfully documented emigration from natal ranges, but settlement in breeding ranges has rarely been observed due to mortalities, shed transmitters and loss of contact. Data available, however, show such a high frequency of emigration by male bears as to suggest the behaviour may be nearly universal. In contrast, few cases of female dispersal have been reported. Summarizing the published data, only 6% of 79 juvenile females emigrated from their natal range (Rogers 1987a; Elowe & Dodge 1989; Beck 1991; Schwartz & Franzmann 1992). Radio-telemetry results from New Mexico corroborated these findings (Costello 2008). Mean distance between the centre of the natal home range and the most recent annual range ( $\geq 3$  years old) was 34.0 km for males but only 2.9 km for females.

The purpose of this study was to quantify the effect of this sex-biased dispersal on the genetic structure of the populations and the potential for close inbreeding. Our working hypothesis was that male-biased dispersal reduces close inbreeding by limiting the spatial overlap of opposite-sex pairs of close relatives. We further hypothesized that the resulting spatial genetic structure would minimize for the need for active inbreeding avoidance (via kin recognition) among potential mates residing near one another. Based on these hypotheses, we made the following specific predictions: (i) relatedness among female pairs would decrease with distance, but relatedness among male pairs and opposite-sex pairs would not differ with distance (or would be less correlated with distance); (ii) closely related opposite-sex pairs would rarely live in close proximity to

each other; and (iii) as a consequence of predictions (i) and (ii), opposite-sex pairs residing near one another would mate at random, relative to relatedness.

## Materials and methods

This paper draws from a larger study primarily designed to investigate natality and survival within two black bear populations in New Mexico (Costello *et al.* 2001). Capture, radio-telemetry monitoring and den investigations were our primary means of obtaining data on individually known bears. Collection and subsequent analyses of DNA samples allowed us to conduct post-hoc investigations of parentage, relatedness and social structure.

### Study areas

We studied bears in two populations separated by ~475 km and tracts of unsuitable habitat. The 310 km<sup>2</sup> Northern Study Area (NSA) was located in the Sangre de Cristo Mountains of northern New Mexico. The 420 km<sup>2</sup> Southern Study Area (SSA) was located in the Mogollon Mountains of west-central New Mexico. Land use and hunting differed between the study areas. The NSA encompassed private and state lands and was adjacent to two towns. Recreation and cattle ranching were the primary land uses. The more remote SSA was within Gila National Forest and was 3–16 km away from the three closest towns. Livestock grazing and timber harvest were primary land uses. With cooperation of private landowners, the entire NSA was closed to bear hunting for study purposes during 1992–1998. State lands within the area had been closed to bear hunting since the 1960s. Beginning in 1998, limited hunting was reestablished on some private lands. The SSA was open to bear hunting throughout the study period. Using population reconstruction, estimated mean density of bears one or more years old was 17.0 bears/100 km<sup>2</sup> in the NSA and 9.4 bears/100 km<sup>2</sup> in the SSA (Costello *et al.* 2001). Dominant habitat types in both areas included pinon–juniper (*Pinus edulis*–*Juniperus* spp.) woodlands; oak–mountain mahogany (*Quercus* spp.–*Cercocarpus* spp.) scrub; ponderosa pine (*Pinus ponderosa*), mixed conifer (*Pseudotsuga menziesii*–*Abies concolor*), aspen (*Populus tremuloides*) and spruce–fir (*Picea engelmannii*–*Abies lasiocarpa*) forests; and meadow of mixed grasses (*Festuca* spp., *Muhlenbergia montana*, *Bouteloua* spp. and *Poa* spp.). Elevations ranged from 2070 m to 3793 m in the NSA and 1750 m to 3035 m in the SSA. Climate varied with elevation, with slightly warmer and drier condition in the SSA. Thirty-year average temperatures were –7 to 0 °C in January and 16 to 21 °C in July in the NSA, and –1 to 5 °C in January and 19 to 24 °C in July in the SSA. The frost-free growing season was 70–190 days in the NSA and 110–230 days in the SSA. Annual precipitation ranged from 37.8 to 41.4 cm in the NSA and 37.6 to 40.4 cm in the SSA, with

most occurring as snowfall or rainfall during July and August (Western Regional Climate Center 2001).

### *Capture and telemetry*

We marked or uniquely identified 516 individual bears (198 F, 290 M, 28 unknown) between September 1992 and June 2000, by means of trapping and den investigations, detailed in Costello *et al.* (2001), Costello *et al.* (2003), and Inman *et al.* 2007). We captured 300 individuals 517 times. Nearly all adult ( $\geq 5$  years) and subadult (2–4 years) females captured ( $n = 99$ ) were radio-marked with collars or ear-tag transmitters, but adult males were radio-marked as needed to maintain a sample of about 10 individuals each year ( $n = 53$ ). Initially, captured subadult males ( $n = 29$ ) were also radio-marked, but this practice was terminated after 1994. Using telemetry to locate dens, we handled or observed 342 bears in dens 683 times to document reproduction and fit or refit collars. Yearling females ( $n = 28$ ) and males ( $n = 30$ ) were radio-marked in the den when accessible. We chemically immobilized adult, subadult, and yearling bears, but handled six- to eight-week-old cubs in the den without immobilization. Handled bears were sexed, measured, and marked with eartags and lip tattoos. We estimated age (based on body size and tooth eruption, wear, and coloration) and extracted a vestigial premolar tooth from most bears ( $n = 292$ ) for age determination using cementum annuli counts (Willey 1974). Radio-marked bears were located from fixed-wing aircraft on a 14-day schedule during the active season (i.e. outside of hibernation). We recorded locations using Universal Transverse Mercator (UTM) grid coordinates to the nearest 0.1 km, on U. S. Geological Survey 7.5-minute maps. We obtained 5723 radio-telemetry locations for 239 bears (127 females, 112 males).

### *Microsatellite DNA analysis*

We collected samples from 430 bears (83% of individuals) to obtain microsatellite genotypes. During all years, we collected tissue samples obtained from punching holes in ears for ear-tagging ( $n = 377$ ). Samples collected before 1998 were frozen, those collected after 1998 were stored in lysis buffer (Longmire *et al.* 1997). In addition, we froze whole blood obtained from bears captured during 1992–1996 ( $n = 50$ ). Beginning in 1997, hairs with fresh follicles were pulled and stored in envelopes with silica dessicant beads ( $n = 320$ ).

We extracted DNA from 1–4 samples for 422 individuals, with a preference for blood or tissue when available (samples were misplaced for eight individuals). We extracted DNA using DNEasy blood and tissue kits (Quiagen, Valencia, California, USA). We numbered DNA extractions separately from bear identification numbers, therefore the process of

genotyping repeated samples from the same bear was blind. We amplified 11 microsatellite loci previously shown to have useful heterozygosity in bear populations: CXX20 (Ostrander *et al.* 1993), G1D, G10L, G10P (Paetkau *et al.* 1995), G10J, G10O (Paetkau *et al.* 1998), UarMu05, UarMu10, UarMu15, UarMu23, and UarMu59 (Taberlet *et al.* 1997). Each polymerase chain reaction (PCR) contained ~50 to 150 ng of extracted DNA. All 11 loci PCRs, with 5' end fluorescently labeled forward primer, were performed using the DNA Engine DYAD thermal cycler (Bio-Rad Laboratories) and visualized with the 3100-Avant Genetic Analyzer (Applied Biosystems). All PCRs contained 1X Gold Buffer, locus-specific MgCl<sub>2</sub> concentration (1.5–2.5 mM), 200 ng/ $\mu$ L bovine albumin serum (BSA), 1.0 mM dNTPs, 2.0  $\mu$ M each of forward-labeled and reverse primers, 1.0 Unit AmpliTaq Gold@ DNA Polymerase (Applied Biosystems), and deionized water for a constant reaction volume of 15  $\mu$ L. Following optimization, the thermal profile consisted of one cycle at 95 °C for 10 min, followed by 45 cycles at 95 °C for 30 s, a locus-specific annealing temperature (48–57 °C) for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 30 min. We combined four loci into two PCR duplexes (Cxx20 with G10O and UarMU05 with UarMU23). PCR products were combined, on the basis of size and fluorescent labeling, into three pools of 3–5 loci each for visualization: consisting of (i) G10L, G10P, G10H, and Mu59; (ii) Cxx20, G10O, G10J, UarMU05, and UarMU23; and (iii) G1D, UarMU10, and UarMU15. All allelic calls (i.e. determinations of the presence and size of an allele) were made using GENEMAPPER 3.7 (Applied Biosystems) with automatic allelic call setup. In addition to automatic allelic calls, all allelic calls were manually checked for accuracy. We discarded entire multilocus genotypes when < eight of 11 loci amplified successfully ( $n = 29$  or 6% of total). With the remaining sample, we calculated a genotyping error rate of 0.01 errors per allele by comparing multilocus genotypes of 41 pairs of samples taken from the same individual. This error rate does not include the potential for alleles to match despite both being incorrect. If errors are independent, this would occur in only one of 10 000 comparisons.

### *Analysis of relatedness and geographical distance*

To examine genetic structure, we estimated relatedness and distance between each pair of adult bears that lived in the same study area at the same time. As our focus was spatial genetic structure in the population of post-dispersal, potentially breeding individuals, we restricted our analyses to pairs of bears that were both  $\geq 4$  years old. Previous analyses indicated that most bears settled into their adult home range by four years of age (Costello 2008), similar to another study (Rogers 1987b). The youngest documented breeding age was three for both males and females (Costello *et al.* 2003; Costello 2008), but successful breeding at this

age was rare [i.e. four of 46 (9%) females, one of 27 (4%) males]. Our unbiased estimate (Garshelis *et al.* 1998) of mean age of first breeding was 4.7 years for females (Costello *et al.* 2003) and 6.5 years for males (Costello 2008). We further restricted analyses to those dyads that were known or presumed to occupy their home ranges during the same year. We presumed that bears captured as adults were present within their home range from age four until the end of the study, unless they were known to have died. This assumption is justified because human-caused mortality was well-documented (i.e. known) and mortality from other sources was rare ( $\leq 0.06/\text{year}$ ; Costello *et al.* 2001). Finally, we restricted analysis to bears located at least once during the period between den emergence and 20 July (pre-mast season), when bears typically stay within, or close to, their established home range. It was not possible to ascertain whether bears captured after this date had breeding ranges within the study area, because bears travel widely in search of food during fall hyperphagia (mast season). We characterized each dyad by sex: female–female, male–male, or female–male. This yielded a sample of 5858 dyads (2473 NSA and 3385 SSA) comprised of 161 individuals (80 F and 81 M; 74 NSA and 87 SSA). Of these dyads, 25% were female–female, 26% were male–male and 49% were female–male.

We estimated a home-range centre for each bear and determined the distance between home-range centres for each dyad of bears. Incremental analysis, to determine change in the estimated home-range centre with sample size, indicated that a minimal sample of pre-mast locations was needed to estimate the home-range centre. For most bears [125 of 161 (78%)], we calculated a 50% kernel home range (Silverman 1986) fit with a smoothing parameter of 3800 m for males and 1800 m for females, based on 6–100 locations ( $\bar{x} = 37.5 \pm 24.1$  SE). These values were the rounded mean of  $h_{\text{ref}}$  for bears with  $\geq 30$  locations (Costello 2008). Most of these ranges were represented by a single contour. When ranges were represented by two, or more rarely three contours, one contour typically contained predominantly pre-mast season locations, while the other(s) typically contained mast season locations. The home-range centre was estimated as the centre coordinates of the kernel contour dominated by pre-mast season locations. For animals known to have dispersed long distances (i.e. those that moved to a new home range  $\geq 20$  km from their natal range; Costello 2008), we used contours containing post-dispersal locations. These analyses were conducted using the Animal Movements 2.0 extension (Hooge & Eichenlaub 2000) developed for the ARCVIEW 3.3 software (Environmental Systems Research Institute, Redlands, California). For the remaining 36 (22%) bears, we had limited location data (only 1–3 locations), either because they were not radio-marked or they shed their radio-transmitter prematurely. We estimated their home-range centre as the arithmetic mean

of their locations, because a kernel estimate was impossible with  $\leq 3$  locations. Patterns in relatedness vs. distance were very similar regardless of whether we included the bears with limited data. However, we chose to include these bears, because it allowed us to boost the overall sample size (which was important when distance was small) and to balance the number of observations between the sexes (since fewer males were radio-marked).

We used ML-Relate (Kalinowski *et al.* 2006) to obtain a maximum likelihood estimate of the coefficient of relatedness,  $r$ , for each dyad. In addition, we also identified the most likely relationship between bears in each dyad. Within this sample of mature bears, we had 32 dyads with verified relationships: six mother–offspring pairs known from field observations; and 10 mother–offspring, 11 father–offspring, one full sibling and four half sibling relationships determined by parentage analysis using CERVUS 3.0 software (Marshall *et al.* 1998; Kalinowski *et al.* 2007; Costello 2008). Although parentage analysis allowed us to identify parent–offspring relationships with 95% confidence, it did not allow us to identify full- or half-sibling relationships unless the parents were included in our sample (which was unlikely for many of the older bears). Using maximum likelihood, ML-Relate determines a single ‘most likely’ relationship for each dyad among four categories: parent–offspring (PO), full sibling (FS), half sibling (HS), or unrelated (U; i.e. all relationships with relatedness lower than half siblings). Unfortunately, the likelihood obtained for the most likely relationship was often not much higher than the likelihood(s) obtained for other categories. ML-Relate provides a simulation method to determine which of the four relationship categories were consistent with genetic data for  $\alpha = 0.05$ . We ran this analysis (using 50 randomizations) and found 85% of dyads had  $> 1$  relationship consistent with the data. Consequently, we used this output to estimate the ‘most distant’ relationship consistent with the data. Neither the most likely nor the most distant categorization appeared to be completely accurate for assessing the true relationship for all dyads. Using the most likely relationship, 78% of our verified relationships were correctly identified, but another 225 dyads were also identified as PO. It was quite unlikely that we failed to identify this many PO relationships using CERVUS, suggesting the most likely relationship was biased toward relationships closer than reality (similar to other estimators; Van Horn *et al.* 2008). Only 3% of our verified relationships were correctly identified using the most distant relationship, suggesting it was biased toward relationships more distant than reality (as expected). Consequently, we examined relationship frequencies among dyads using both the most likely and the most distant relationships, and we presumed the true relationship frequencies fell somewhere between them. This dual analysis allowed us to explicitly test the sensitivity of our inferences to uncertainties inherent in all current

**Table 1** Number of alleles ( $k$ ), number of individuals typed ( $n$ ), observed heterozygosity ( $H_O$ ), and expected heterozygosity ( $H_E$ ) for 11 microsatellite loci sampled from black bears in the Northern Study Area (NSA) and Southern Study Area (SSA), New Mexico, 1992–2000. No significant deviations from Hardy–Weinberg equilibrium were observed ( $P > 0.05$  for  $\chi^2$  tests at all loci)

Locus	NSA				SSA			
	$k$	$n$	$H_O$	$H_E$	$k$	$n$	$H_O$	$H_E$
Cxx20	6	201	0.57	0.60	6	189	0.32	0.30
G10J	7	212	0.72	0.72	7	194	0.70	0.71
G10L	8	211	0.84	0.80	8	193	0.80	0.79
G10O	5	209	0.40	0.42	2	193	0.13	0.13
G10P	7	210	0.51	0.50	6	193	0.57	0.55
G1D	6	211	0.80	0.76	6	192	0.76	0.72
Mu05	5	210	0.35	0.41	5	193	0.43	0.42
Mu10	7	210	0.68	0.67	6	194	0.62	0.68
Mu15	4	212	0.58	0.61	3	195	0.44	0.46
Mu23	5	212	0.51	0.51	2	195	0.35	0.36
Mu59	7	212	0.65	0.65	8	195	0.71	0.71

methods of estimating relationships. Finally, where relationships were known from other data, we substituted these known relationships into both categories (most likely and most distant). This methodology was consistent with the suggestions of Van Horn *et al.* (2008), who recommended assigning ranges of possible kinship derived from a combination of pedigree data and pairwise genetic estimators of relatedness, to overcome the limitations in accuracy and precision of current methods.

We tested for relationships between distance and relatedness by estimating the correlation coefficient between the natural log of distance and  $r$  for each sex category. We used a randomization method akin to the Mantel test (Mantel 1967) to obtain a significance level, which accounts for the unavoidable lack of independence in dyadic data (i.e. many dyads shared one bear in common). The  $r$  column was subjected to 10 000 random permutations, a distribution of correlation coefficients was obtained for the randomized data, and the proportion of values more extreme than the observed value was determined (i.e. the  $P$ -value). Second, we examined mean  $r$  and relationship frequencies for all dyads within three distance categories: 3 km, 6 km and 35 km. Three km was the rounded mean distance between an adult female and her mother (i.e. female natal dispersal distance). Six km was the rounded mean distance between mating pairs (i.e. mating distance) as determined by parentage analysis. Forty km was the rounded mean distance between male offspring and their mother (i.e. male natal dispersal distance). We calculated mean and 95% confidence intervals for  $r$  within these distance categories to compare across sexes and study areas. We determined frequencies of relationships within these distance categories and used

the  $\chi^2$  statistic to test for differences by sex and study area. To determine whether bears selected mates according to relatedness within the pool of spatially available mates, mean  $r$  and relationship frequencies observed for actual mating pairs (determined by parentage analysis) were compared to those expected from opposite-sex dyads occurring within the mean mating distance. Analyses were conducted using *SPSS* 11.5 and the *PopTools* 2.7 add-in (Hood 2006) for Microsoft Excel 2000.

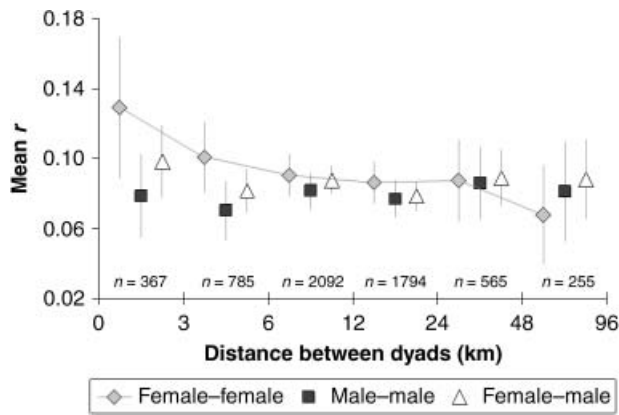
All analyses were run separately for each study area and then with data pooled from both areas. In this context, 'pooled' does not mean we considered dyads with one bear from the NSA and one bear from the SSA. It means that data from the two sites were combined after within-site analyses. Where patterns were the same on both study areas, we report only the pooled result.

## Results

Our full microsatellite data set consisted of 8–11 loci typed for 407 bears; 212 bears from the NSA and 195 bears from the SSA (Table 1). This represented 79% of bears identified during the study and 95% of bears from whom samples were collected. Genotyping failed for 15 bears and samples were misplaced for eight bears. No significant deviations from Hardy–Weinberg equilibrium were observed for any of the 11 loci ( $P > 0.05$ ). Among our sample of  $\geq 4$ -year-old bears, we obtained relatedness coefficients for pairs ranging from 0.0 to 0.79, with a mean of 0.080 in the NSA and a mean of 0.089 in the SSA. The most likely relationships (MLR) were 79% U, 15% HS and 6% PO/FS, while the most distant relationships (MDR; see Methods) were 92% U, 7% HS and  $\leq 1\%$  PO/FS.

### Genetic structure

As predicted, relatedness was negatively correlated with log-distance among female dyads ( $r = -0.08$ ,  $P = 0.003$ ) but was not correlated with log-distance among male dyads ( $r = 0.004$ ,  $P = 0.44$ ) or among female–male dyads ( $r = -0.02$ ,  $P = 0.19$ ; Fig. 1). Mean  $r$  was higher for female dyads than for male dyads or female–male dyads when considering all pairs within 3 km ( $F_{2,364} = 2.5$ , 0.08), all pairs within 6 km ( $F_{2,1149} = 4.8$ , 0.008) and all pairs within 35 km ( $F_{2,5305} = 5.0$ , 0.007). When examined in more details, these differences were attributable to a higher frequency of close relationships among female dyads (Fig. 2). Whether we classified dyads according to the MLR or the MDR, we detected significantly more PO/FS relationships among female dyads than either male dyads or female–male dyads within the same distance ( $\chi^2 P \leq 0.03$ ). The size of this effect decreased with distance, although the statistical significance of the difference increased due to sample size;  $n = 367$  within 3 km,  $n = 1152$  within 6 km and  $n = 5308$  within 35 km. On an individual



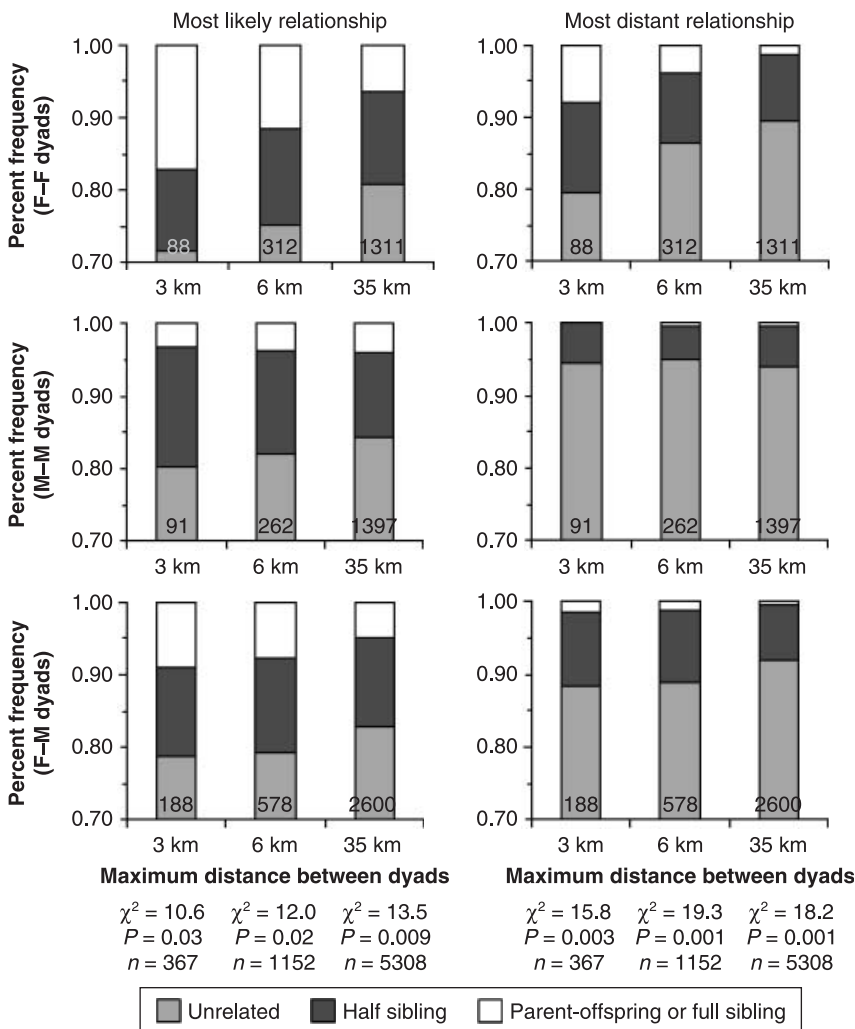
**Fig. 1** Mean ( $\pm$  95% CI) relatedness coefficient ( $r$ ) among dyads of black bears ( $\geq 4$  years old), by sex, within quasi-natural logarithmic increments of distance. Based on individual observations, relatedness was negatively correlated with log-distance among female dyads ( $r = -0.08$ ,  $P = 0.003$ ) but was not correlated with log-distance among male dyads ( $r = 0.004$ ,  $P = 0.44$ ) or among female-male dyads ( $r = -0.02$ ,  $P = 0.19$ ).

basis, a lower proportion of bears had a male relative residing within 6 km than had a female relative residing within that distance (Fig. 3). However, when study areas were examined separately, the difference was absent or less pronounced in the SSA.

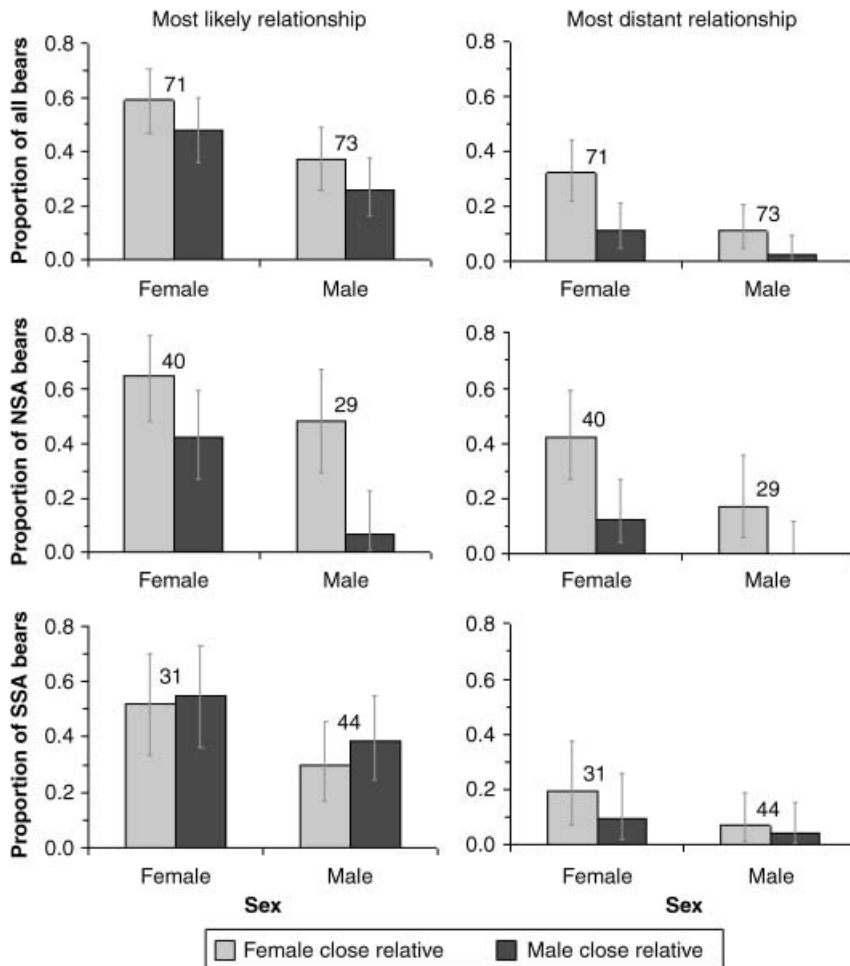
*Inbreeding avoidance*

Within the mean mating distance, most opposite-sex dyads were unrelated: 79% based on the MLR or 89% based on the MDR (Fig. 4). Only 8% (MLR) or 1% (MDR) of these dyads consisted of PO/FS pairs. When we examined bear ages in these dyads and assumed an age difference of  $\geq 4$  years between parents and their offspring, then father-daughter relationships appeared to be most common (45% using MLR or 88%, using MDR), followed by full sibling relationships (39%, using MLR or 13%, using MDR) and mother-son relationships (15%, using MLR).

Among 56 actual mating pairs, mean  $r$  was 0.067, and this value did not differ from the mean for all opposite-sex



**Fig. 2** Percent frequency of relationships among dyads of all black bears ( $\geq 4$  years old), within three cumulative distance categories: (top) female-female dyads; (centre) male-male dyads; and (bottom) female-male dyads. More than one relationship was possible for most dyads; therefore, dyads were first classified according to the most likely relationship (left) and then according to the most distant relationship (right). Based on field data and parentage analysis, 3 km was the rounded mean female dispersal distance, 6 km was the rounded mean distance between mating pairs, and 35 km was the rounded mean male dispersal distance. Note that the  $y$ -axis begins at 0.70 to better illustrate differences among groups. Sample sizes appear within each bar and test statistics refer to vertical comparisons of relationship frequencies among sexes within distance categories.



**Fig. 3** Proportion ( $\pm$  95% CI) of individual black bears ( $\geq 4$  years old), by sex, that had a close relative (parent–offspring or full sibling) residing within the mean mating distance of 6 km: (top) all bears from both study areas; (centre) bears from the Northern Study Area (NSA); and (bottom) bears from the Southern Study Area (SSA). More than one relationship category was possible for most dyads; therefore, dyads were first classified according to the most likely relationship (left) and then according to the most distant relationship (right). Sample sizes appear above bars.

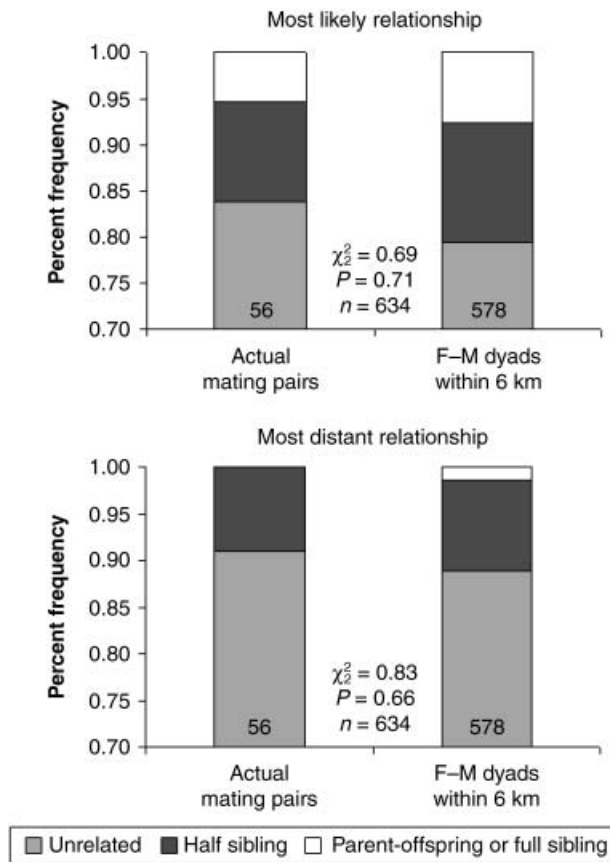
dyads residing with the mean mating distance, 0.087 ( $t = 0.51$ ,  $P = 0.61$ ). As expected if mating within the pool of spatially available mates was random, most of the actual mating pairs were unrelated (84% using MLR or 91% using MDR). Only three dyads (5%) consisted of PO/FS pairs using MLR; none using MDR. Again, assuming an age difference of  $\geq 4$  years between parents and their offspring, then two of these three PO/FS mating pairs appeared to be full sibling relationships and one appeared to be a father–daughter relationship. We observed no mother–son relationships among mating pairs. Relationship frequencies did not differ between mating pairs and opposite-sex dyads residing within the mean mating distance (Fig. 4), using either criterion for assigning relationships ( $\chi^2 P \geq 0.66$ ).

## Discussion

Our analyses revealed sex differences in the spatial genetic structure of the study populations consistent with male-biased dispersal. As predicted, mean relatedness and proportion of relatives decreased as a function of distance among female pairs, while little change was observed with

distance among male pairs or opposite-sex pairs. In addition, females living in close proximity were, on average, more closely related than males living near one another. These patterns were apparent in both study areas and are consistent with previous descriptions of black bear ecology and behaviour (Rogers 1987a,b; Elowe & Dodge 1989; Beck 1991; Schwartz & Franzmann 1992). A similar spatial genetic structure was found in Scandinavian populations of brown bears (*Ursus arctos*; Støen *et al.* 2005), where dispersal was also male-biased (Støen *et al.* 2006; Zedrosser *et al.* 2007).

Nonetheless, the sex effect in the genetic structure was smaller than would be expected if male dispersal and female philopatry were nearly absolute, as was observed among our sample of radio-marked offspring ( $n = 22$ ; Costello 2008). If all, or nearly all, females settled near their natal range, we would expect most females to have a close female relative residing nearby. But when examining individual bears, we found that only 33–59% of females had another female PO/FS relative within 6 km. Thus, the potential for kin-structuring to affect social evolution in female black bears, such as that postulated for brown bears (Støen *et al.* 2005; Zedrosser *et al.* 2007), was weaker than



**Fig. 4** Percent frequency of relationships for mating pairs of black bears (determined by parentage analysis) compared to those for opposite-sex dyads ( $\geq 4$  years old) residing within the mean mating distance of 6 km. More than one relationship was possible for most dyads; therefore, dyads were first classified according to the most likely relationship (top) and then according to the most distant relationship (bottom). Note that the *y*-axis begins at 0.70 to better illustrate differences among groups. Sample sizes appear within each bar and test statistics refer to horizontal comparisons of relationship frequencies between mating pairs and opposite-sex dyads residing within 6 km.

one might have previously assumed. Similarly, if all, or nearly all, males dispersed  $\geq 20$  km from their natal range, we would expect few, if any, to have a close male relative residing nearby. But as much as 26% of males had another male PO/FS relative residing within 6 km. These and other patterns in the proportion of bears with neighbouring relatives suggested that the sex bias in dispersal was probably not as high as our field results implied. They also suggested that the sex bias was less pronounced in the SSA, owing primarily to differences among males.

At first glance, the lower-than-expected proportions of females with close female relatives living nearby suggested that some females might have dispersed away from their natal ranges. However, a closer examination showed that the estimates were consistent with the relatively low repro-

ductive potential of bears in New Mexico. Mean natality and survival rates (Costello *et al.* 2001, 2003) indicated that females surviving to the age of 4 years would recruit only about 1.4 female offspring (also surviving to age four) during an average life span. With this low reproductive potential, it would be unlikely that any female would have more than a few closely related female neighbours and many females would have none. Both adult female survival and natality were slightly higher in the NSA than in the SSA, apparently due to the hunting closure and more consistent oak production (Costello *et al.* 2001, 2003). Thus, NSA females would recruit about 1.8 female offspring during their lifetime, while SSA females would recruit about 1.0. This difference in recruitment is a reasonable explanation for the higher proportion of females in the NSA that had closely related female relatives residing within 6 km (43% vs. 19% based on MDR,  $\chi^2_2 = 4.2$ ,  $P = 0.04$ ; Fig. 4).

The substantial proportion of males that had closely related males residing nearby may indicate a lack of dispersal by some males, or at least dispersal distances shorter than those we observed in the field. If inbreeding avoidance was the sole driver of male dispersal, it would be reasonable to assume that rates of male dispersal would be density-independent (e.g. Zedrosser *et al.* 2007), but our data suggest that males in the lower-density SSA dispersed less often or shorter distances than males in the NSA. Higher density has been hypothesized to increase dispersal rates by compelling more individuals to emigrate to reduce resource or mate competition (Greenwood 1980; Dobson 1982; Waser 1985).

We suspect that mate competition among males was a factor in dispersal of black bears. Results of our paternity analysis indicated that higher densities and lower turnover of mature males ( $\geq 7$  years old) lessened the chances of mating for young males (Costello 2008), probably making areas with low male density more appealing for establishment of a home range by a dispersing male. Density estimates indicated bears were 1.7 to 2.0 times more numerous in the NSA than the SSA during our study period (Costello *et al.* 2001), although mature male density was not different. Estimated densities in both study areas were relatively low ( $\leq 19$  bears/100 km<sup>2</sup>) and likely well below carrying capacity; densities of  $> 30$  bears/100 km<sup>2</sup> have been estimated for nearby Arizona populations (LeCount 1982; Waddell & Brown 1984). Although slightly higher natality in the NSA (Costello *et al.* 2003) may account for some of this difference in density, it is likely that hunting (before and during the study period) reduced the SSA population below carrying capacity to a much larger degree than the NSA, where some hunting closures were in effect since the 1960s. We suspect some males in the SSA responded to low density by remaining near their natal range, where competition from other males was low. Higher mortality among both sexes might reduce the likelihood that philopatric,



opposite-sex pairs would simultaneously survive to reproductive age. Thus, in heavily exploited populations, philopatric males may benefit from the scarcity of males near their natal range, without suffering the cost of close inbreeding. Similar to our finding, Ji *et al.* (2001) found that mean relatedness of male brushtail possums (*Trichosurus vulpecula*) was higher (and more similar to that of females) in populations recovering from intense mortality than in undisturbed populations. They suggested that this was explained by the short-distance dispersal of related males into the disturbed area from the edge of the undisturbed area.

Despite some evidence that the sex bias in dispersal was, perhaps, lower than expected, the potential for close inbreeding was still low. We estimated that 79–89% of the 578 opposite-sex dyads residing within the mean mating distance were unrelated (meaning their relatedness was lower than that of half siblings). Only between 1% and 7% of these dyads appeared to involve PO/FS relationships. Although some individuals had an opposite-sex close relative residing nearby, unrelated neighbours far outnumbered relatives. We found no evidence that bears were actively avoiding inbreeding in their selection of mates from this nearby pool of relatives and nonrelatives. The frequencies of probable relationships among mating pairs and those among dyads within the mean mating distance were remarkably similar.

Avoidance of close kin, particularly paternal kin, as potential mates would likely require a relatively sophisticated form of kin recognition in bears. In a review of paternal kin discrimination studies, Widdig (2007) focused on two primary mechanisms for kin recognition: familiarity and phenotypic matching. The solitary behaviour of bears does not provide a social context in which fathers and their offspring become familiar with each other, nor does it offer much opportunity for siblings born in different litters to gain familiarity. Promiscuous mating by female bears would also reduce a male's ability to ascertain the parentage of offspring produced by a female mate. Therefore, paternal kin recognition in bears would likely require phenotypic matching (to self or familiar kin) by means of a cue, such as odour or appearance. Little is known about the costs or effectiveness of these phenotypic recognition mechanisms. However, Hain & Neff (2006) studied helping behaviour among bluegill sunfish (*Lepomis macrochirus*) nest mates and estimated the cost of self-referent kin recognition. They found that the cost was high enough for its expression to be inhibited among those individuals for whom most other nest mates were full siblings (~80%). In contrast, kin recognition was expressed among those individuals with few full siblings in the nest (~20%), because the cost of misplaced helping behaviour was even higher than the cost of kin recognition. Just as the preponderance of close kin alleviated the need for kin recognition in sunfish, the preponderance of non-kin probably alleviated the need for

kin recognition in bears. Our results indicated mean  $r$  was only 0.087 among opposite sex pairs residing within the average mating distance, and more than 80% of pairs were unrelated. With this low potential for close inbreeding, presumably a result of sex-biased dispersal, there would appear to be little need for active inbreeding avoidance in bears, especially if mechanisms for distinguishing kin bore substantial fitness costs. Other studies have found that where dispersal minimized inbreeding, inbreeding avoidance through mate choice was weak or absent (Ims & Andreassen 1991; Peters & Michiels 1996; Banks *et al.* 2005; Hansson *et al.* 2006).

Among the relatively few closely related (i.e. PO or FS), opposite-sex pairs residing near each other, father–daughter relationships were the most common, as expected, given female-biased philopatry. Our paternity analyses showed that male reproductive success was dominated by a fraction of intermediate-aged bears (Costello 2008), indicating that most males would have a relatively short reproductive tenure. This short tenure would also minimize the need for kin recognition, as few males would be expected to successfully compete for mates once they reached an age old enough to have fathered a reproductively mature female. Nonetheless, we were able to document individual males fathering litters separated by as many as nine years (Costello 2008), more than enough time for a female offspring to mature. Thus, the individuals most likely to engage in close inbreeding would be the most successful males and their daughters. For these males, secondary dispersal might provide a means to reduce the likelihood of mating with a daughter, such as that observed in swift foxes (*Vulpes velox*; Kamler *et al.* 2004); however, we found no evidence of males abandoning their established home ranges after the age of six years (Costello 2008).

In summary, our results show that high rates of male dispersal and female philopatry combine to create a spatial genetic structure that generates low rates of inbreeding and little need for kin discrimination among potential mates. Thus, our evidence supports the hypothesis that inbreeding avoidance is achieved by means of male-biased dispersal in black bears. Our results also suggest that the general pattern of male-biased dispersal is modified by competition for mates or resources.

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