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Abstract

Mating with close kin can lead to inbreeding depression through the expression of recessive deleterious alleles and loss of heterozygosity. Mate selection may be affected by kin encounter rate, and inbreeding avoidance may not be uniform but associated with age and social system. Specifically, selection for kin recognition and inbreeding avoidance may be more developed in species that live in family groups or breed cooperatively. To test this hypothesis, we compared kin encounter rate and the proportion of related breeding pairs in noninbred and highly inbred canid populations. The chance of randomly encountering a full sib ranged between 1–8% and 20–22% in noninbred and inbred canid populations, respectively. We show that regardless of encounter rate, outside natal groups mates were selected independent of relatedness. Within natal groups, there was a significant avoidance of mating with a relative. Lack of discrimination against mating with close relatives outside packs suggests that the rate of inbreeding in canids is related to the proximity of close relatives, which could explain the high degree of inbreeding depression observed in some populations. The idea that kin encounter rate and social organization can explain the lack of inbreeding avoidance in some species is intriguing and may have implications for the management of populations at risk.

Keywords: *Canis lupus*, dispersal, kin recognition, sexual selection, *Vulpes lagopus*

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Introduction

The ability of individuals to recognize kin has been demonstrated in a wide variety of species (e.g. Hepper 1986; Pusey & Wolf 1996; Hauber & Sherman 2001;

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Mateo 2003, 2004). For example, Belding's ground squirrels (*Spermophilus beldingi*) and ring-tailed lemurs (*Lemur catta*) use olfactory signals to precisely estimate kinship, even among distant relatives (Mateo 2002; Charpentier *et al.* 2008, 2010). However, the ability to recognize kin as a mechanism to avoid inbreeding varies among species (e.g. Pusey & Wolf 1996; Mateo 2002, 2004). Specifically, mate selection mechanisms designed to avoid

inbreeding are more likely to develop in species where kin encounter each other as adults (Pusey & Wolf 1996). For example, house mice (*Mus musculus*) often live at high density in family-based social groups where kin encounter rate is high, and kin recognition mechanisms are thus required to avoid mating with kin. In contrast, the Macedonian mouse (*Mus macedonicus*) lives at much lower densities where kin encounter is considerably less frequent than in the house mouse (Sherborne *et al.* 2007). Beynon *et al.* (2007) showed that urinary proteins are highly variable in the house mouse but lack variability in the Macedonian mouse. Variation in urinary proteins may provide a mechanism for kin recognition in these mice and show greater variation in the species where kin encounter rate is higher (Barnard *et al.* 1991; Sherborne *et al.* 2007).

An important function of kin recognition is to provide a mechanism by which inbreeding can be avoided. Inbreeding avoidance and its consequences have been documented in many species (reviewed by Pusey & Wolf 1996; Crnokrak & Roff 1999; Keller & Waller 2002; Edmands 2006). The detrimental effects of inbreeding are well known for captive populations, and vertebral malformations have been recently documented in inbred wolf populations (Räikkönen *et al.* 2006, 2009). However, the detrimental effects of inbreeding in wild populations are less clear because some species show no inbreeding avoidance and no ill effect associated with inbreeding (e.g. Table 1 in Pusey & Wolf 1996; Reindeer (*Rangifer tarandus*): Holand *et al.* 2007; great reed warbler (*Acrocephalus arundinaceus*): Hansson *et al.* 2007; great tit (*Parus major*): Szulkin *et al.* 2009; New Zealand saddleback (*Philesturnus carunculatus*) and robin (*Petroica australis*): Jamieson *et al.* 2009). More recent reviews (Crnokrak & Roff 1999; Keller & Waller 2002) suggest that many populations in the wild are not able to avoid inbreeding or limit its phenotypic effects and that in some cases, the genetic load may be effectively purged (Crnokrak & Barrett 2002; Hagenblad *et al.* 2009). In addition, sex-biased dispersal, common in many animals including canids (Moehlman 1989; Geffen *et al.* 1996), does not result in a complete partition of male and female relatives, and so individuals may have mechanisms to recognize kin and avoid inbreeding.

Richard *et al.* (2009) showed that within a lizard species, monogamous females of intermediate ages did not mate with genetically similar partners. On the other hand, polyandrous females, generally from the young and the old age classes, did not discriminate partners according to relatedness. Thus, inbreeding avoidance was not uniform but associated with age and social system. Selection for kin recognition and inbreeding avoidance may be more developed in species that live in family groups and breed cooperatively. However, in such cases, individuals can reduce inbreeding without

Table 1 Kin (full- or half-sib level) encounter rate in four populations of grey wolves (95% CI in parenthesis)

Population	Full-sib level ($r \geq 0.50$)	Half-sib level ($r \geq 0.25$)
Denali National Park (AK; $N_m = 62$, $N_f = 67$)		
All male–female pairs ($n = 4155$)	0.049 (0.043, 0.049)	0.184 (0.184, 0.184)
Between packs ($n = 3249$)	0.042 (0.035, 0.042)	0.171 (0.170, 0.171)
Within packs ($n = 220$)	0.250 (0.197, 0.316)	0.554 (0.483, 0.607)
Superior National Forest (MN; $N_m = 35$, $N_f = 20$)		
All male–female pairs ($n = 271$)	0.059 (0.034, 0.096)	0.133 (0.096, 0.180)
Between packs ($n = 245$)	0.012 (0.002, 0.035)	0.065 (0.037, 0.106)
Within packs ($n = 26$)	0.500 (0.295, 0.700)	0.769 (0.544, 0.911)
Isle Royale National Park (MI; $N_m = 18$, $N_f = 15$)		
All male–female pairs ($n = 693$)	0.196 (0.171, 0.197)	0.307 (0.280, 0.308)
Between packs ($n = 490$)	0.131 (0.103, 0.160)	0.231 (0.196, 0.268)
Within packs ($n = 210$)	0.352 (0.297, 0.421)	0.495 (0.424, 0.567)
Yellowstone National Park (1995–1999; WY; $N_m = 50$, $N_f = 49$)		
All male–female pairs ($n = 2370$)	0.043 (0.036, 0.053)	0.132 (0.118, 0.133)
Between packs ($n = 2184$)	0.015 (0.010, 0.020)	0.077 (0.066, 0.077)
Within packs ($n = 266$)	0.267 (0.221, 0.328)	0.586 (0.523, 0.637)
Yellowstone National Park (2000–2004; WY; $N_m = 97$, $N_f = 65$)		
All male–female pairs ($n = 6305$)	0.030 (0.026, 0.030)	0.148 (0.148, 0.148)
Between packs ($n = 5808$)	0.017 (0.013, 0.020)	0.116 (0.116, 0.117)
Within packs ($n = 497$)	0.187 (0.149, 0.220)	0.519 (0.485, 0.553)

Kin encounter rate is calculated for all pair combinations, all possible pairs between packs and all possible pairs within packs. N_m and N_f are the number of males and females, respectively, and n is the number of male–female pair combinations evaluated.

developing kin recognition skills by selecting partners that are not from their natal group. Following these lines, Jamieson *et al.* (2009) argued that if encounter rates between close kin are low and kin screening mechanisms are associated with significant costs (e.g. additional search time for appropriate mate or missed opportunity costs associated with attaining a territory), then selection for inbreeding avoidance will be weak. Cost may be greatest when the breeding habitat is saturated, and additional search efforts for an appropriate mate can potentially lead to the loss of breeding opportunities (e.g. loss of the breeding territory to others). Consequently, when kin encounter rate is low, the probability of inbred mating is also low regardless of mate searching intensity. Likewise, when kin encounter probability is high, the probability of mating with a relative may not be easily reduced by greater search intensity for nonkin. However, investment in mate screening is beneficial for intermediate kin encounter rates. Jamieson *et al.* (2009) concluded that investment in breeding avoidance via kin discrimination is social system–dependent and that inbreeding avoidance should be more frequently observed in cooperative breeding species, relative to individual-pairing monogamous species, owing to the difference in kin encounter rate.

Canids are territorial, living in pairs or family groups, and all are generally monogamous, with a single breeding pair in a social unit (Moehlman 1989; Geffen *et al.* 1996; Mech 1999). In many cases, breeding is cooperative with nonbreeding helpers assist by provisioning and guarding the pups. Both sexes emigrate, but females tend to delay dispersal. We selected two canid species, the grey wolf (*Canis lupus*) and the arctic fox (*Vulpes lagopus*), which are known to disperse great distances with registered records of over 1000 km (e.g. Fritts 1983; Tarroux *et al.* 2010). The structure of canid social systems predicts a high kin encounter rate within natal groups and low kin encounter rate outside natal groups. Our aim was to test the hypothesis of Jamieson *et al.* (2009), which implies that mate selection is affected by kin encounter rate. We examined whether mates are randomly selected outside natal groups and whether inbreeding is avoided within natal groups, by determining the proportion of related pairs in populations with various levels of inbreeding. Inbred populations are expected to have a larger proportion of related individuals relative to noninbred populations. We hypothesized that in known inbred populations of canids (i.e. Isle Royale National Park and southern Scandinavia), kin encounter rate outside natal groups would be higher relative to noninbred populations, where it is expected to be low. Consequently, if individuals screen for relatives, we would expect more intense selection against random mating in inbred populations,

where kin encounter rate is high also outside the natal group. Alternatively, if individuals are selected only to avoid mating with natal group members, we would predict that mate selection in inbred populations would not deviate from that expected by chance, regardless of the kin encounter rate. To test our hypothesis, we compared kin encounter rate and proportion of related breeding pairs in the highly inbred wolf population of Isle Royale National Park (Wayne *et al.* 1991; Räikkönen *et al.* 2009; Adams *et al.* 2011) and the noninbred populations of Denali National Park, Yellowstone National Park and Superior National Forest (Smith *et al.* 1997; vonHoldt *et al.* 2008, 2010). Likewise, for the arctic fox, we compared the inbred populations in Scandinavia (e.g. Dalèn *et al.* 2006; Nystrom *et al.* 2006) and the noninbred population in north-western Iceland (Geffen *et al.* 2007; Norèn *et al.* 2009).

Methods

We compiled microsatellite data for four populations of grey wolves and three populations of arctic foxes. Data for populations in Denali National Park and Superior National Forest were taken from Smith *et al.* (1997). This data set consisted of 130 adult individuals from at least 19 different packs in Denali National Park (Alaska) and 33 adults from 11 packs in Superior National Forest (Minnesota), which were screened using 20 polymorphic microsatellites (Smith *et al.* 1997). Data for the wolf population from Isle Royale National Park (Michigan) consisted of 56 adults from five packs. These samples were screened using eight polymorphic microsatellites (Adams *et al.* 2011). Finally, we used 10 years of data for Yellowstone National Park wolves (vonHoldt *et al.* 2008). During 1995–1996, 31 wolves from Canada were translocated to Yellowstone. At the end of 1999, at least 118 wolves in about 10 packs were present in the greater Yellowstone area. During 2000–2004, the population increased further and levelled off at about 250 wolves in 16 packs (Smith *et al.* 2004). The period of 1995–1999 was a time of population establishment, whereas during 2000–2004, the wolf population reached its maximum density. Moreover, prey availability per wolf was high after introduction but decreased in later years as the wolf population increased in number. Because of the differences in environmental and social conditions between these two periods, and because most of the animals present earlier were no longer there during the later period, we split the data into two 5-year sets. These two data sets were composed of 103 adults sampled during 1995–1999 and 172 adults sampled during 2000–2004. All Yellowstone National Park wolves were screened using 26 polymorphic microsatellites (vonHoldt *et al.* 2008, 2010). Smith *et al.*

(1997) showed that relatedness values stabilized using ≥7–9 microsatellites. All the above-mentioned wolf populations had been closely studied for many years, and pack assignment (i.e. breeder vs. nonbreeding helper), age, and social status were known for most individuals (e.g. Mech 1986; Gese & Mech 1991; Lehman *et al.* 1992; Meier *et al.* 1995; Mech *et al.* 1998; Peterson *et al.* 1998; vonHoldt *et al.* 2008).

Our arctic fox populations comprised 49 adults from Hornvik in north-west Iceland, 25 adults from Ammarnäs to Finnmark in northern Scandinavia and 38 from the Vålådalen Nature reserve in southern Scandinavia. The arctic fox population in Scandinavia has been under threat since the early 1900s, and its breeding performance has been intensively studied (e.g. Angerbjörn *et al.* 1991; Dalèn *et al.* 2006; Meijer *et al.* 2008). The long-term study on Scandinavian arctic foxes involved direct observations at breeding dens and radiotelemetry, which enabled detailed information on all breeding and natal individuals at both study sites. The arctic foxes in Hornvik were also intensively studied in the past (Hersteinsson *et al.* 2000). The behavioural data presented here were collected over 6 years (2002–2007) by some of us (MK, RH, PH, AA, LD, EF and EG), using direct observations on 4–5 breeding dens annually, where foxes were individually marked with coloured ear tags and some were fitted with GPS-based collars (GPS-3300SL, Lotek Wireless; Tellus mini, Televilt). All arctic fox populations were screened using 10 polymorphic microsatellite loci following the methods in Dalèn *et al.* (2006). The microsatellite data set for the population in Hornvik is the only one not previously published (Table S1).

Relatedness between all possible male–female pair combinations in each population was calculated using the program ML-RELATE (Kalinowski *et al.* 2006). The program calculates maximum likelihood estimates of relatedness (r; Wagner *et al.* 2006), which are considered more accurate than estimates produced by other algorithms (Milligan 2003). The program is designed to discriminate among three common pedigree relationships: unrelated (U; $r = 0$), half-siblings (HS; $r = 0.25$) and full-siblings/parent–offspring (FS; $r = 0.50$). This is carried out by calculating the likelihood of R, which is the probability of observing the ratio between the genotypes of the two individuals having a relationship r at the given homologous locus. The probability values for each locus are multiplied across loci to yield the likelihood of the relationship, $L(R)$, and the relatedness relationships are defined by the highest likelihood (Wagner *et al.* 2006). Simulations by Wagner *et al.* (2006, table 8) showed that the algorithm is efficient in assigning the correct relatedness categories (83% for eight loci, 87% for 10 loci and 95% for 20 loci). This program also adjusts the relatedness values to accommodate null alleles (for details see Wagner *et al.* 2006).

We estimated the probability of an individual to pair with a related mate (i.e. kin encounter rate) by calculating the proportion of full-sib pairs ($r \geq 0.5$; FS) or at least half-sib pairs ($r \geq 0.25$; FS + HS) out of all possible male–female pair combinations in each population. In this approach, we assume that within a population, each male can hypothetically pair with any female, a reasonable assumption given the daily movement capabilities of dispersing wolves and foxes (90 and 40 km/day for fox and wolf, respectively; Audet *et al.* 2002; Mech & Cluff 2009; Tarroux *et al.* 2010), and the size of our study sites (longest distance across: 60–500 km). Because wolves live in packs composed of highly related family members (Lehman *et al.* 1992; Smith *et al.* 1997), we repeated the aforementioned calculations after the exclusion of all within-pack male–female pair combinations. To calculate the probability that the observed ratio between the numbers of related and unrelated known pairs in each population differs from the expected under the random mating model, we used a randomization procedure. We randomly sampled the number of known pairs from the list of all possible pairs in each population and counted the number of related pairs within the sample. We repeatedly sampled, with replacement, 10 000 times from each population. We used the program resample (M. Wood, University of Portsmouth; http://userweb.port.ac.uk/~woodm/nms/resample.htm) to calculate the probability of obtaining all possible numbers of related pairs in a sample and the 95% confidence interval (CI) for that distribution.

Results

We calculated kin encounter rate as the proportion of the number of related pair combinations relative to the total number of possible pairs in the population. This proportion can be viewed as the probability of selecting a mate by random, i.e. a full-sib ($r \geq 0.5$) or at least a half-sib ($r \geq 0.25$). Tables 1 and 2 show the kin (FS or HS level) encounter rate in four populations of grey wolves and three populations of arctic foxes, respectively. Encounter rate with an HS mate was 3.0 (±1.3) times more likely than with an FS mate in the wolf populations and 2.3 (±0.8) times more likely with an HS than an FS mate in the fox populations (paired test by permutations over all populations, $P = 0.004$; Table 1). For the wolf populations, we calculated kin encounter rate for all male–female combinations, all possible male–female pairs between packs and all possible male–female pairs within packs. Kin encounter rates within packs were significantly higher than between

Table 2 Kin (full- or half-sib level) encounter rate in three populations of arctic foxes (95% CI in parenthesis)

Population	Full-sib level ($r \geq 0.50$)	Half-sib level ($r \geq 0.25$)
Hornvik, north-western Iceland ($N_m = 27$, $N_f = 18$; $n = 486$)	0.072 (0.051, 0.098)	0.216 (0.183, 0.252)
Northern Scandinavia ($N_m = 11$, $N_f = 14$; $n = 154$)	0.084 (0.046, 0.140)	0.195 (0.140, 0.267)
Southern Scandinavia ($N_m = 18$, $N_f = 20$; $n = 360$)	0.222 (0.184, 0.276)	0.328 (0.283, 0.381)

N_m and N_f are the number of males and females, respectively, and n is the number of male–female pair combinations evaluated.

different packs in all wolf populations (15.8-fold greater likelihood for FS (paired test by permutations, $P = 0.031$) and 5.9-fold greater for HS ($P = 0.038$), respectively; Table 1). Kin encounter rates in neighbouring packs were similar to those of nonadjoining packs in Denali NP (Fisher's exact test; FS: $P = 0.803$, HS: $P = 0.770$) and in Yellowstone NP (1995–1999; FS: $P = 0.830$, HS: $P = 0.235$; Fig. 1). However, in Yellowstone NP during 2000–2004, kin encounter rates were significantly lower in nonadjoining packs relative to adjoining packs (FS: $P < 0.0001$, HS: $P < 0.0001$; Fig. 1).

We calculated the relatedness between known alpha pairs in four wolf populations (10, 6, 7, 18 and 22 pairs in Denali NP, Superior NF and Isle Royale NP, and Yellowstone NP (1995–1999) and Yellowstone NP (2000–2004), respectively) and between known pairs in three arctic fox populations (11, 6 and 6 pairs in Hornvik, north Scandinavia and south Scandinavia, respectively). For the wolf populations, the number of related pairs out of all the known pairs was not significantly different than expected by random mate selection. This result was apparent for both FS and HS encounter rates, and for all possible female–male pair combinations or only for the combinations between packs (Figs 2 and 3). The only exception was Isle Royale NP, where the number of HS-related pairs was significantly higher (HS = 4; $P = 0.045$) than expected by random mate selection (Fig. 3). Under random mating, the CI showed that in most wolf populations, the expected number of pairs related at full-sib level ranged from 0 to 2. CI for half-sib level pairs varied considerably more (Figs 2 and 3). In contrast, the observed number of related pairs, given the within-pack kin encounter rate (Table 1), was considerably lower than expected by random mate selection in all wolf populations except for Isle Royale NP. This outcome was evident for both FS and HS (Denali NP: $P = 0.061$ and $P = 0.074$; Superior NF: $P = 0.015$ and $P = 0.0002$; Isle Royale NP: $P = 0.293$ and $P = 0.271$; Yellowstone NP 1995–1999: $P = 0.076$ and $P = 0.0001$; Yellowstone NP 2000–2004: $P = 0.011$ and $P < 0.0001$). Lastly, in all the Arctic fox populations, the number of observed related pairs out of all known pairs was not significantly different than expected by a random mate selection (Fig. 4).

Discussion

Our results are in agreement with the predictions of Jamieson *et al.* (2009) for monogamous social systems, typical in canids. In outbred populations, there was 1–8% chance of encountering a full sib outside the natal pack. This low encounter rate means random mating will generally result in unrelated pairs. We showed that the ratio between related and unrelated breeding pairs in both wolf and arctic fox non-inbred populations was not significantly different from the expected by random mating, as predicted by Jamieson *et al.* (2009). Interestingly, the ratio between related and unrelated pairs also did not deviate from that expected by random mating in the inbred populations, even though the kin encounter rate in these populations was much higher (about 20%). However, the observed number of related pairs was considerably lower than expected by random mating within packs. Our results suggest that pack members are excluded as mates, as was also found by Smith *et al.* (1997) for Denali NP and Superior NF wolves, and by vonHoldt *et al.* (2008), based on a detailed pedigree analysis of Yellowstone NP wolves. Our results also suggest that individuals may pair indiscriminately, with respect to relatedness, with any potential mate outside their natal group, regardless of kin encounter rate.

Given the small number of pairs in many of our populations, and the fact that zero is the lower 95% CI for interpack pairs in all populations, the issue of statistical power to detect inbreeding avoidance may be a concern. If kin encounter rate is low, the overall population inbreeding level will be similarly low regardless of whether canids actively avoid mating with related individuals as mates or mate indiscriminately outside their natal pack. For example, in Denali NP, where kin encounter rate is about 4%, zero-related pairs are still probable in a random sample of up to 70 pairs ($P \sim 0.05$). Our only support for kin recognition is from the Isle Royale NP population where the number of related pairs between packs was more than expected by chance (HS; Fig. 3b). Wolves in this population preferred half-sibs as mates, a result that contradicts active inbreeding avoidance.
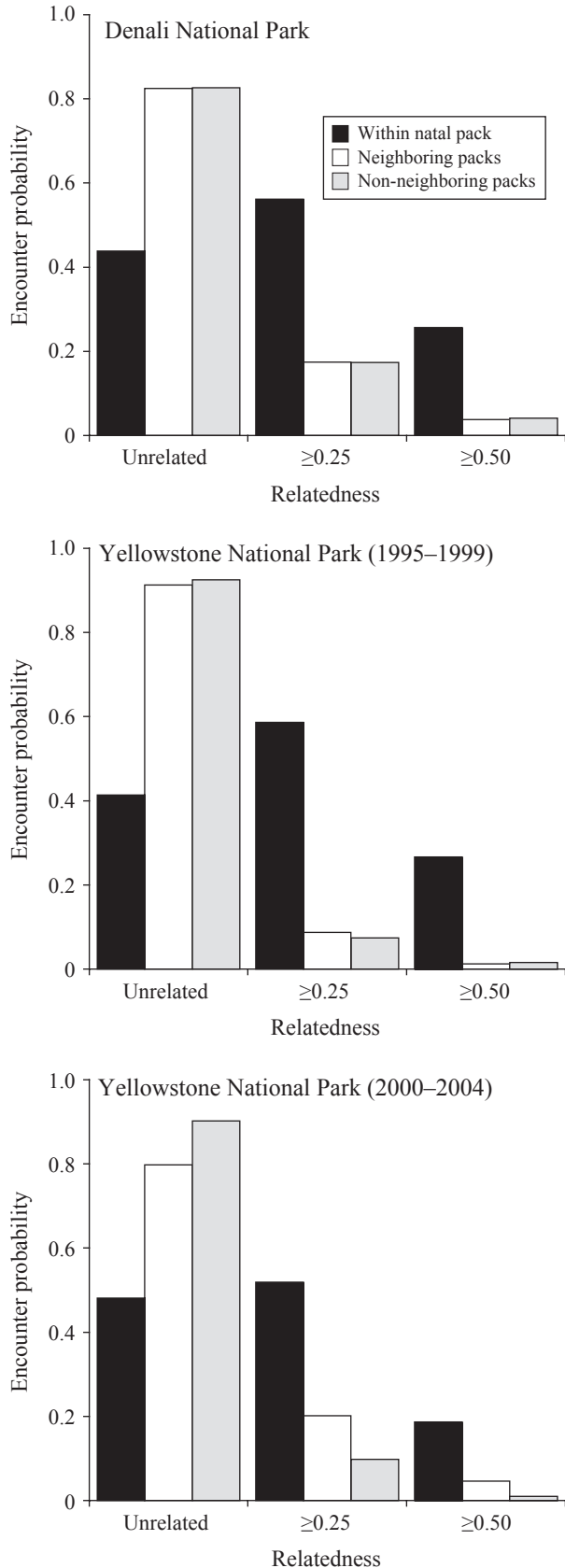
Why should canids mate indiscriminately with non-natal group members? Two demographic issues are relevant here. First, grey wolf and arctic fox populations were historically large and contiguous and spanned vast areas (e.g. Vila *et al.* 1999; Nystrom *et al.* 2006). Under such conditions, kin encounter rate and inbreeding are always expected to be low outside the natal pack. Furthermore, as long as pack members are avoided as mates, the selective force against inbreeding is weak because the likelihood of encountering a kin by chance is low, and thus, the consequences of inbreeding affect only a small portion of the population (Jamieson *et al.* 2009). This may explain why random mating, in respect to relatedness, is still exercised in canid populations that have recently become inbred. Second, Jamieson *et al.* (2009) suggest that the associated cost of screening for nonrelated mates might have dire consequences in some social systems. Canids are highly territorial and in stable or expanding large populations, mated pairs may occupy most of the suitable area. Without a territory, individuals are more likely to have decreased reproductive fitness. The territory provides secured resources for the parents and juvenile offspring and is maintained year-round. The crucial step for a young male/female disperser in search of a breeding opportunity is first to acquire and maintain a territory, through a variety of mechanisms (e.g. usurpation, inheritance, occupation of an unclaimed territory). Under the usurpation scenario, an individual can take over a territory, replace the original breeder and mate with the original breeder's mate, to which it is probably unrelated. In the case of unclaimed territory, the individual must remain in it and defend its boundaries once it is attained. Leaving in search of a potential mate (e.g. under the inheritance scenario) will most likely result in the territory being taken over by another individual. Under the unclaimed territory scenario, territory holders should pair with any immigrant potential mate that enters their territory, especially so when kin encounter rate is low. However, the inheritance of a territory increases the chance of inbreeding. Furthermore, territory holders leaving their territory in search of a non-natal mate bear an additional risk (i.e. cost) of aggressive encounters with neighbouring pairs while traversing their territory. The
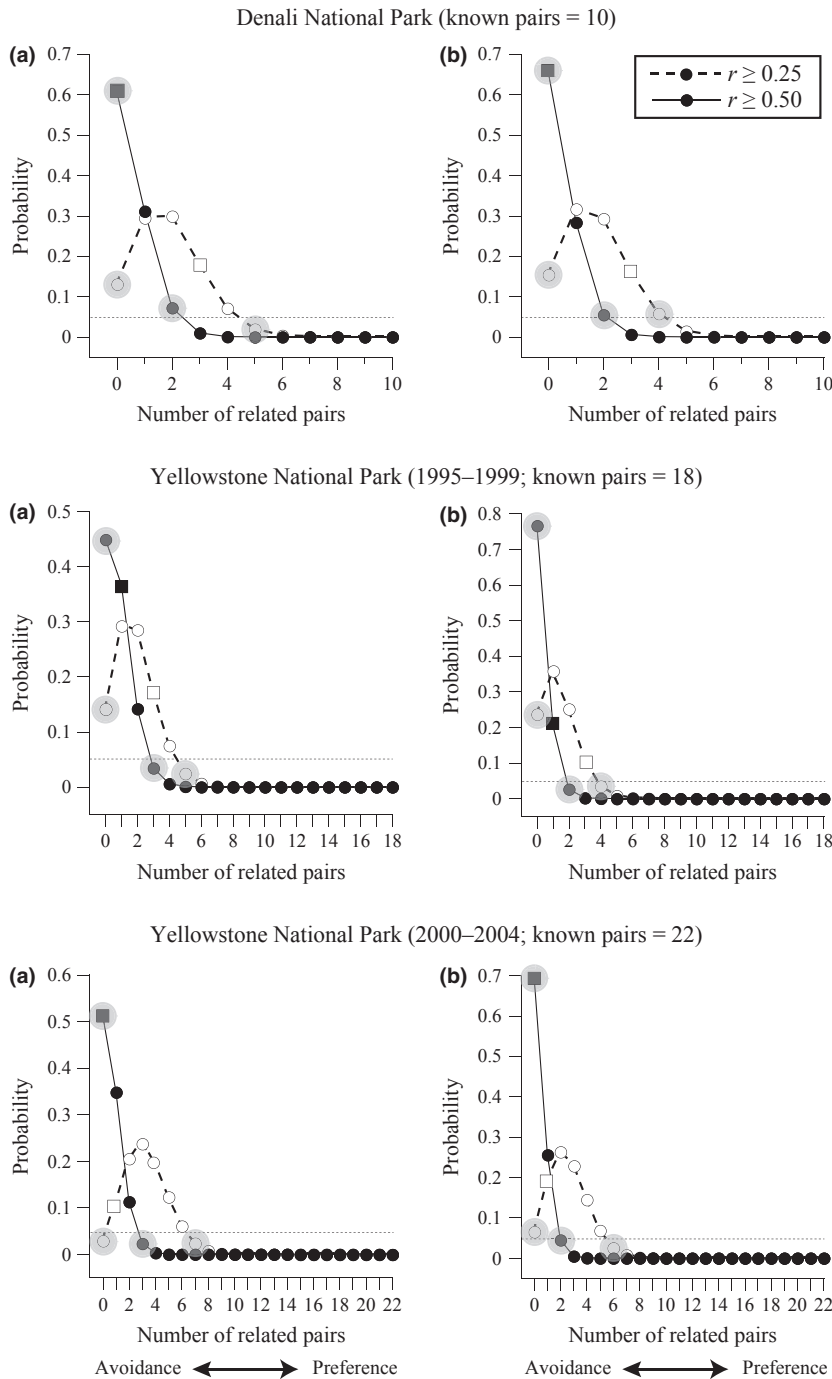
Fig. 2 The probability of observing a number of related breeding pairs out of the total number of known pairs recorded in three wolf populations (empty circle for at least half-sib level ($r \geq 0.25$) relatives and filled circle for at least full-sib level ($r \geq 0.50$) relatives). For each site (Denali National Park and Yellowstone National Park) and time frame (1995–1999, 2000–2004), two alternative models were considered: (a) all possible mates are equally probable, and (b) only mates outside the natal pack are selected. Square symbols indicate the observed number of related pairs. The dotted horizontal line represents $P = 0.05$, and the grey circles indicate upper and lower 95% confidence intervals for the number of related pairs possible.

salient point of these scenarios or any other scenario is that securing a territory may have higher priority over the avoidance of inbreeding. The high cost of territory maintenance alone may promote random mating even when kin encounter rate outside the natal group is moderately high. Moreover, the costs involved with prioritizing territory attainment over inbreeding may fluctuate greatly between populations and between years within a population. For example, in Arctic fox

populations where prey is cyclic (e.g. lemmings), the likelihood of successful breeding is much higher during lemming peak years (Tannerfeldt & Angerbjörn 1998; Elmhagen *et al.* 2000). Consequently, these lemming peak years may promote even less selection against relatives as potential mates because the probability of attaining a suitable territory is high and the number of unpaired individuals to select from is lower.
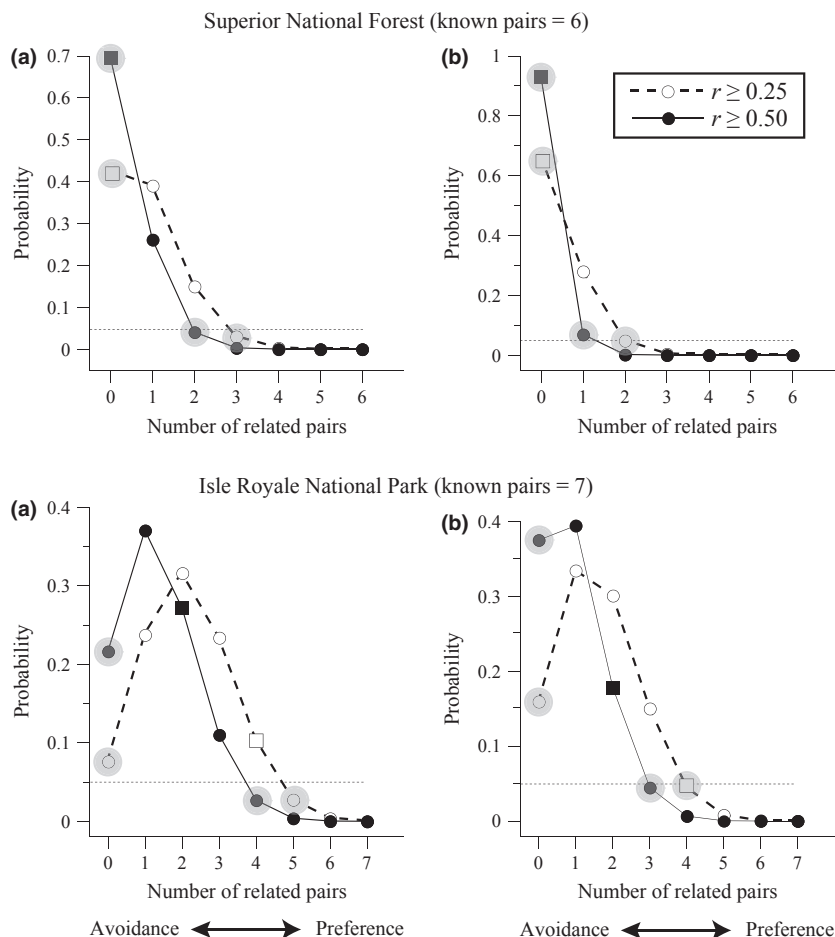
Superior National Forest (known pairs = 6)

Isle Royale National Park (known pairs = 7)

Avoidance ◄——► Preference

Avoidance ◄——► Preference

Fig. 3 The probability of observing a number of related breeding pairs out of the total number of known pairs recorded in two wolf populations (empty circle for at least half-sib level ($r \geq 0.25$) relatives and filled circle for at least full-sib level ($r \geq 0.50$) relatives). For each site (Superior National Forest and Isle Royale National Park), two alternative models were considered: (a) all possible mates are equally probable, and (b) only mates outside the natal pack are selected. Square symbols indicate the observed number of related pairs. The dotted horizontal line represents $P = 0.05$, and the grey circles indicate upper and lower 95% confidence intervals for the number of related pairs possible.

An additional concern is whether sufficient time has elapsed to allow inbreeding avoidance to evolve in small populations. We know in the domestic dog that new breeds radically different in morphology and behaviour can be developed rapidly within 10–20 generations (e.g. vonHoldt *et al.* 2010). This fact only demonstrates the potential of canids for rapid change under intense selection. Moreover, the wolves of Isle Royale reached the island during the late 1940s and have been there for at least 20 generations (generation time is 3 years; Peterson *et al.* 1998). The Arctic fox population in southern Scandinavia has become highly inbred over the last 30 years (~15 generations). These time frames are probably sufficiently long to develop new traits (e.g. Hill & Kirkpatrick 2010). Furthermore, strong selection against deleterious recessive alleles has been documented and demonstrated experimentally in small populations (e.g. Hagenblad *et al.* 2009; Bouzat 2010). Thus, the indiscriminate mating outside the natal pack in canids may not be related to population size or the time available to evolve new mating preferences but more to the presence of insufficient selective pressure to drive the change in mating preference.

In this study, we do not claim that canids select their mates randomly or that kin recognition and inbreeding avoidance do not occur at any level. As in many other species, grey wolves and Arctic foxes may screen potential mates according to phenotypic traits, previous breeding experience, genetic variability or other desirable traits. We suggest only that in respect to relatedness, when kin encounter rate is low, selection against kin may not be a better strategy than selecting a mate by random from outside the natal pack. The idea that kin encounter rate and social organization can explain the lack of inbreeding avoidance in some species is intriguing. Furthermore, the association between weak selection against inbreeding and social organization (i.e. kin encounter rate) may have implications for the management of populations at risk (Jamieson *et al.* 2009). The possible lack of discrimination against close relatives (outside packs) in small populations implies that the rate of inbreeding in canids may be higher than in species that actively avoid inbreeding (e.g. Liberg *et al.* 2005). Given basic information for a certain species (e.g. pairwise relatedness, social organization), the latency to inbreeding depression can be predicted and integrated
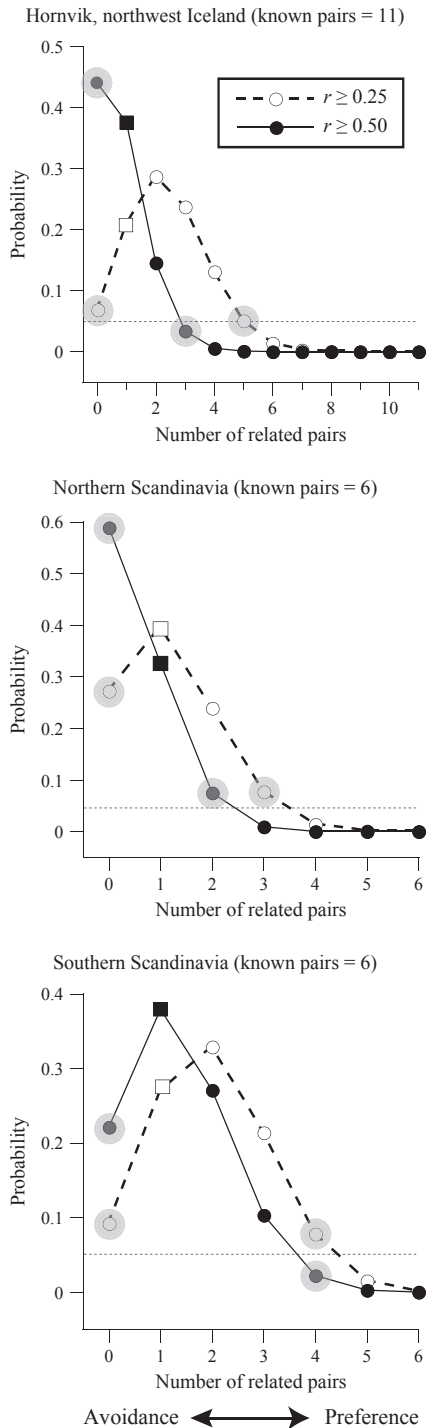
Fig. 4 The probability of observing a number of related Arctic fox breeding pairs out of the total number of known pairs recorded in three Arctic fox populations (empty circle for at least half-sib level ($r \geq 0.25$) relatives and filled circle for at least full-sib level ($r \geq 0.50$) relatives). Square symbols indicate the observed number of related pairs. The dotted horizontal line represents $P = 0.05$, and the grey circles indicate upper and lower 95% confidence intervals for the number of related pairs possible.

into population management efforts. In practice, the readily available behavioural and genetic data from long-term studies on a variety of wildlife can be used for estimating kin encounter rate and predicting the level of inbreeding avoidance in a wide range of social systems.

Acknowledgements

References

Adams JR, Vucetich LM, Hedrick PW, Peterson RO, Vucetich JA (2011) Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. *Proceedings of the Royal Society B*, DOI: 10.1098/rspb.2011.0261.

Angerbjörn A, Arvidson B, Norèn E, Stromgren L (1991) The effect of winter food on reproduction in the arctic fox, *Alopex lagopus*—a field experiment. *Journal of Animal Ecology*, **60**, 705–714.

Audet AM, Robbins CB, Larivière S (2002) *Alopex lagopus*. *Mammalian Species*, **713**, 1–10.

Barnard CJ, Hurst JL, Aldhous P (1991) Of mice and kin—the functional significance of kin bias in social behavior. *Biological Reviews of the Cambridge Philosophical Society*, **66**, 379–430.

Beynon RJ, Turton MJ, Robertson DHL *et al.* (2007) Urinary lipocalins in Rodenta: is there a generic model? In: *Chemical Signals in Vertebrates*, Vol. 11 (eds Hurst JL, Beynon RJ, Roberts SC and Wyatt T), pp. 37–50. Springer, New York.

Bouzat JL (2010) Conservation genetics of population bottlenecks: the role of chance, selection, and history. *Conservation Genetics*, **11**, 463–478.

Charpentier MJE, Boulet M, Drea CM (2008) Smelling right: the scent of male lemurs advertises genetic quality and relatedness. *Molecular Ecology*, **17**, 3225–3233.

Charpentier MJE, Crawford JC, Boulet M, Drea CM (2010) Message 'scent': lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. *Animal Behaviour*, **80**, 101–108.

Crnokrak P, Barrett SCH (2002) Purging the genetic load: a review of the experimental evidence. *Evolution*, **56**, 2347–2358.

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. *Heredity*, **83**, 260–270.

Dalèn L, Kvaloy K, Linnell JDC *et al.* (2006) Population structure in a critically endangered arctic fox population: does genetics matter? *Molecular Ecology*, **15**, 2809–2819.

Edmands S (2006) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, **16**, 463–475.

Elmhagen B, Tannerfeldt M, Verucci P, Angerbjörn A (2000) The arctic fox—an opportunistic specialist. *Journal of Zoology*, **251**, 139–149.

Fritts SH (1983) Record dispersal of a wolf from Minnesota. *Journal of Mammalogy*, **64**, 166–167.

Geffen E, Gompper ME, Gittleman JL, Luh HK, MacDonald DW, Wayne RK (1996) Size, life-history traits, and social organization in the canidae: a reevaluation. *American Naturalist*, **147**, 140–160.

Geffen E, Waidyaratne S, Dalèn L *et al.* (2007) Sea ice occurrence predicts genetic isolation in the Arctic fox. *Molecular Ecology*, **16**, 4241–4255.

Gese EM, Mech LD (1991) Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. *Canadian Journal of Zoology*, **69**, 2946–2955.

Hagenblad J, Olsson M, Parker HG, Ostrander EA, Ellegren H (2009) Population genomics of the inbred Scandinavian wolf. *Molecular Ecology*, **18**, 1341–1351.

Hansson B, Jack L, Christians JK *et al.* (2007) No evidence for inbreeding avoidance in a great reed warbler population. *Behavioral Ecology*, **18**, 157–164.

Hauber ME, Sherman PW (2001) Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends in Neurosciences*, **24**, 609–616.

Hepper PG (1986) Kin recognition: functions and mechanisms a review. *Biological Reviews of the Cambridge Philosophical Society*, **6**, 63–93.

Hersteinsson P, Björnsson TT, Unnsteinsdóttir ER, Ólafsdóttir AH, Sigthórsdóttir H, Eiríksson T (2000) The Arctic fox in Hornstrandir: number of dens occupied and dispersal of foxes out of the reserve. *Náttúrufrædingurinn*, **69**, 131–142 (In Icelandic with English Summary).

Hill WG, Kirkpatrick M (2010) What animal breeding has taught us about evolution. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 1–19.

Holand O, Askim KR, Roed KH, Weladji RB, Gjostein H, Nieminen M (2007) No evidence of inbreeding avoidance in a polygynous ungulate: the reindeer (*Rangifer tarandus*). *Biology Letters*, **3**, 36–39.

Jamieson IG, Taylor SS, Tracy LN, Kokko H, Armstrong DP (2009) Why some species of birds do not avoid inbreeding: insights from New Zealand robins and saddlebacks. *Behavioral Ecology*, **20**, 575–584.

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576–579.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, **17**, 130–241.

Lehman N, Clarkson P, Mech LD, Meier TJ, Wayne RK (1992) A study of the genetic-relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. *Behavioral Ecology and Sociobiology*, **30**, 83–94.

Liberg O, Andrén H, Pedersen H-C *et al.* (2005) Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biology Letters*, **1**, 17–20.

Mateo JM (2002) Kin-recognition abilities and nepotism as a function of sociality. *Proceedings of the Royal Society of London B*, **269**, 721–727.

Mateo JM (2003) Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy*, **84**, 1163–1181.

Mateo JM (2004) Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici*, **41**, 729–745.

Mech LD (1986) Wolf population in the central Superior National Forest, 1967–1985. *USDA Forest Service, North Central Forest Experiment Station Research Paper*, **270**, 1–6.

Mech LD (1999) Alpha status, dominance, and division of labor in wolf packs. *Canadian Journal of Zoology*, **77**, 1196–1203.

Mech LD, Cluff HD (2009) Long daily movements of wolves, *Canis lupus*, during pup rearing. *Canadian Field-Naturalist*, **123**, 68–69.

Mech LD, Adams LG, Meier TJ, Burch JW, Dale BW (1998) *The Wolves of Denali*. University of Minnesota Press, Minneapolis, MN.

Meier TJ, Burch JW, Mech LD, Adams LG (1995) Pack structure and genetic relatedness among wolf packs in a naturally-regulated population. In: *Ecology and Conservation of Wolves in a Changing World* (eds Carbyn LN, Fritts SH and Seip DR), pp. 293–302. Canadian Circumpolar Institute, Edmonton, AB.

Meijer T, Norén K, Hellström P, Dalén L, Angerbjörn A (2008) Estimating population parameters in a threatened arctic fox population using molecular tracking and traditional field methods. *Animal Conservation*, **11**, 330–338.

Milligan BG (2003) Maximum-likelihood estimation of relatedness. *Genetics*, **163**, 1153–1167.

Moehlman PD (1989) Intraspecific variation in canid social systems. In: *Carnivore Behavior, Ecology and Evolution* (ed. Gittleman JL), pp. 143–163. Cornell University Press, Ithaca, NY.

Norèn K, Angerbjörn A, Hersteinsson P (2009) Population structure in an isolated Arctic fox, *Vulpes lagopus*, population: the impact of geographical barriers. *Biological Journal of the Linnean Society*, **97**, 18–26.

Nystrom V, Angerbjörn A, Dalèn L (2006) Genetic consequences of a demographic bottleneck in the Scandinavian arctic fox. *Oikos*, **114**, 84–94.

Peterson RO, Thomas NJ, Thurber JM, Vucetich JA, Waite TA (1998) Population limitation and the wolves of Isle Royale. *Journal of Mammalogy*, **79**, 828–841.

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, **11**, 201–206.

Räikkönen J, Bignert A, Mortensen P, Fernholm B (2006) Congenital defects in a highly inbred wild wolf population (*Canis lupus*). *Mammalian Biology*, **71**, 65–73.

Räikkönen J, Vucetich JA, Peterson RO, Nelson MP (2009) Congenital bone deformities and the inbred wolves (*Canis lupus*) of Isle Royale. *Biological Conservation*, **142**, 1025–1031.

Richard M, Losdat S, Lecomte J, de Fraipont M, Clobert J (2009) Optimal level of inbreeding in the common lizard. *Proceedings of the Royal Society of London B*, **276**, 2779–2786.

Sherborne AL, Thom MD, Paterson S *et al.* (2007) The genetic basis of inbreeding avoidance in house mice. *Current Biology*, **17**, 2061–2066.

Smith D, Meier T, Geffen E *et al.* (1997) Is inbreeding common in wolf packs? *Behavioral Ecology*, **8**, 384–391.

Smith DW, Stahler DR, Guernsey DS (2004) *Yellowstone Wolf Project—Annual Report*. Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Szulkin M, Zelazowski P, Nicholson G, Sheldon BC (2009) Inbreeding avoidance under different null models of random mating in the great tit. *Journal of Animal Ecology*, **78**, 778–788.

Tannerfeldt M, Angerbjörn A (1998) Fluctuating resources and the evolution of litter size in the arctic fox. *Oikos*, **83**, 545–559.

Tarroux A, Berteaux D, Bety J (2010) Northern nomads: ability for extensive movements in adult arctic foxes. *Polar Biology*, **33**, 1021–1026.

Vila C, Amorim IR, Leonard JA *et al.* (1999) Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology*, **8**, 2089–2103.

vonHoldt BM, Stahler DR, Smith DW, Earl DA, Pollinger JP, Wayne RK (2008) The genealogy and genetic viability of reintroduced Yellowstone grey wolves. *Molecular Ecology*, **17**, 252–274.

vonHoldt BM, Stahler DR, Bangs EE *et al.* (2010) A novel assessment of population structure and gene flow in grey wolf populations of the Northern Rocky Mountains of the United States. *Molecular Ecology*, **19**, 4412–4427.

Wagner AP, Creel S, Kalinowski ST (2006) Estimating relatedness and relationships using microsatellite loci with null alleles. *Heredity*, **97**, 336–345.

Wayne RK, Lehman N, Girman D *et al.* (1991) Conservation genetics of the endangered Isle Royale gray wolf. *Conservation Biology*, **5**, 41–51.

Data accessibility

Sample locations and microsatellite data: Summary statistics for the Arctic fox population in Hornvik, Iceland are provided in Table S1 (Supporting Information). Microsatellite data for all populations are provided as a Supporting information (Data S1, Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Number of individuals (n), number of alleles (N_{allele}), and expected heterozygosity (H_{exp}) for the 10 polymorphic microsatellite used for screening the arctic fox population in Hornvik, Iceland.

Data S1 Microsatellite data used in this study.

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