

Monozygotic Twin Wolves with Divergent Life Histories

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ABSTRACT. Genetic evidence for monozygotic (identical) twinning in mammalian species is rare in the literature. Here we report what may be the first pair of monozygotic twins identified in a wild caniform carnivore, the grey wolf (*Canis lupus*). One of these individuals remained in its natal population of Banks Island, Northwest Territories, Canada, while its twin migrated across the polar sea ice to the mainland. This suggests divergent life history strategies in genetically identical individuals, making this incidence of twinning particularly interesting.

Key words: wolf, *Canis lupus*, monozygotic twin, identical twin, Banks Island, dispersal, migration, life history

RÉSUMÉ. Il est rare que la documentation fasse état de preuves génétiques à l'égard de jumeaux monozygotes (identiques) chez les espèces de mammifères. Ici, nous faisons mention de ce qui pourrait être la première paire de jumeaux monozygotes identifiée chez un carnivore caniforme sauvage, le loup gris (*Canis lupus*). Un de ces individus est resté au sein de sa population natale de l'île Banks, dans les Territoires du Nord-Ouest, au Canada, tandis que son jumeau a migré jusqu'à la terre ferme au moyen de la glace polaire. Cela laisse entrevoir des stratégies de cycle biologique différentes chez des individus génétiquement identiques, ce qui rend cette gémellité particulièrement intéressante.

Mots clés : loup, *Canis lupus*, jumeau monozygote, jumeau identique, île Banks, dispersion, migration, cycle biologique

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Monozygotic (identical) twins have been extensively studied in humans (e.g., Hrubec and Robinette, 1984) but are rarely documented in other mammals (Gleeson et al., 1994). In fact, we could find no published record confirming monozygotic twinning in any wild carnivore species. Here we present what may be the first evidence of such twinning in a wild caniform carnivore, the grey wolf (*Canis lupus*).

We collected 2025 wolf tissue samples from hunters, fur auction houses, and museums. The sampling distribution spanned most of the North American Arctic. Tissue was stored frozen and extracted using a DNeasy tissue protocol (QIAGEN, Germany). We amplified 14 microsatellite loci using the polymerase chain reaction (PCR) and primers originally designed for domestic dogs (CPH5, CPH16, Fredholm and Wintero, 1995; CXX140, CXX173, CXX250, CXX251, CXX377, Ostrander et al., 1993; CXX618, CXX671, CXX733, CXX745, CXX758, CXX781, CXX2079, Mellersh et al., 1997). DBX and DBY, pseudoautosomal markers for molecular sex identification, were also amplified from each sample (Seddon, 2005). PCR amplification, gel electrophoresis conditions, and analysis of genotypes followed previous studies (Seddon, 2005; Carmichael et al., 2007a), and genotypes are provided in Carmichael et al. (2007a).

Matching genotypes were identified using the Excel Microsatellite Toolkit (Park, 2001). Our database contained 101 pairs of wolves with identical genotypes, but 100 of these pairs most likely resulted from sampling the same individual more than once (in most cases, one sample had been obtained from a fur house and its match directly from a hunter). The genetic identity of the 101st pair, male wolves TU9291 and SH9201, was of particular interest. Wolf TU9291 was sampled in Tuktoyaktuk in the Cape Bathurst region (mainland Northwest Territories, Canada) on 19 February 1992, while SH9201 was collected in Sachs Harbour, Banks Island (Northwest Territories, Canada) nine months later.

Assignment tests, developed by Paetkau et al. (1995), were performed using the Doh calculator developed by J. Brzustowski (available at <http://www2.biology.ualberta.ca/jbrzusto/Doh.php>). In this analysis, the probability that each individual's genotype would arise is calculated for each population on the basis of local allele frequencies. Individuals are then "assigned" to the population in which their genotypes are most likely to occur: their presumptive population of origin (Paetkau et al., 1995). Results indicate that the potential twins share a genotype that originated on Banks Island, rather than in a mainland wolf population (Fig. 1), implying that TU9291 migrated from

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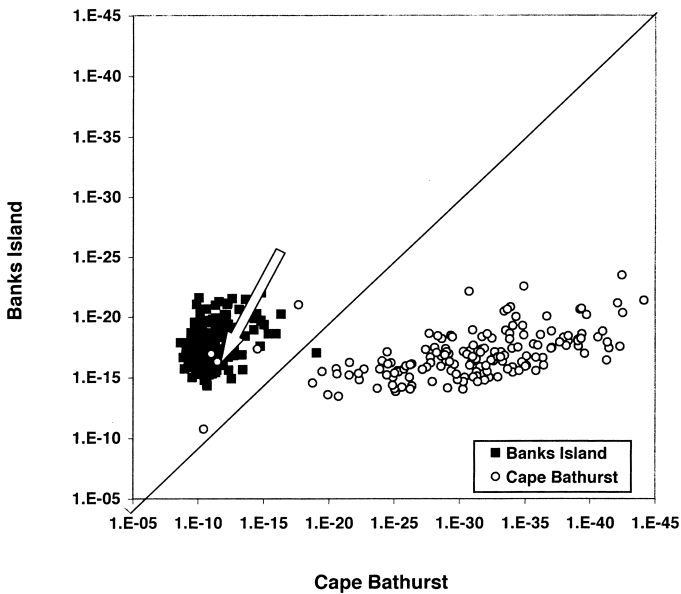


FIG. 1. Assignment test results for two northern Canadian wolf populations. Symbol colour represents sampling population of each individual. Wolves are plotted according to the probability their genotype would arise on Banks Island, the sampling location of SHS9201, or on Cape Bathurst, home of the mainland wolf population in which TU9291 was found (Cape Bathurst is a subregion of the Western Barrens wolf genetic cluster, sensu Carmichael et al., 2007a). The arrow indicates the superimposed white and black points corresponding to these putative monozygotic twin wolves.

Banks Island to the mainland after freeze-up of sea ice between these regions. This migration represents a straight-line movement of at least 375 km.

Four factors suggest that genetic identity of wolves TU9291 and SH9201 is not simply due to error. The samples were submitted to biologists nine months apart, in separate towns, by separate local hunters, and thus could not have been confused during initial collection and processing. Both samples consisted of tissue taken from whole skulls, eliminating the possibility that a hunter could mistakenly submit two samples from a single wolf. The sample identification numbers are visually distinct, reducing the risk of mix-up. Finally, the 14-locus genotypes used here were a composite of data from Carmichael et al. (2001) and from amplifications of additional loci performed using fresh DNA extractions of the original tissue (Carmichael et al., 2007a); laboratory error producing spurious identity is therefore unlikely. Hence, we suggest that TU9291 and SH9201 are two distinct and genetically identical wolves.

We used allele frequencies from Banks Island, the putative population of origin, to calculate probabilities of identity for the particular genotype shared by TU9291 and SH9201. Given one known individual of this genotype, the chance that a second, randomly chosen unrelated individual would be identical was $3.5E-12$. At $3.6E-05$, the chance identity of non-twin siblings is more likely than identity of unrelated wolves, but would still be unexpected given the estimated census size of 200 individuals in this population (Carmichael et al., 2001).

Since Banks Island wolves are less variable than their mainland counterparts (Carmichael et al., 2007b), a mating between related individuals could also produce offspring that are genetically indistinguishable at the loci used here without being monozygotic twins. However, because 7 of 14 loci in this particular genotype are heterozygous, the chance that a sibling mating would produce identical, non-twin offspring was $4.25E-12$ (Futuyma, 2005). In our Banks Island sample, the next closest match consisted of two pairs of individuals, each differing at 2/14 loci. Therefore, while some close relatives may be included in our study, we suggest the heterozygosity of TU9291 and SH9201's genotype makes identity through inbreeding unlikely. Furthermore, Carmichael et al. (2007b) showed that inbreeding is rare or absent in this population.

In 1999, Neff et al. (1999) found one set of verified monozygotic twins during pedigree analysis of approximately 200 domestic dogs (*Canis familiaris*). The occurrence of one set of monozygotic twin wolves in a sample of over 2000 individuals is therefore reasonable, and seems the most likely explanation for the genetic identity of TU9291 and SH9201.

Most interesting of all is the observation that these apparent identical twins experienced divergent life histories, with one individual remaining in its natal island population while the other migrated over sea ice to the mainland. Bayesian estimation suggests a total, bi-directional migration rate of 1.76% between Banks Island and all northern mainland wolf populations (Western Woods; Forest; Western Barrens, including the Cape Bathurst region; Eastern Barrens; and Atlantic; Carmichael et al., 2007b), underscoring the unusual nature of this event.

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