Conservation Genetics of the Endangered Isle Royale Gray Wolf

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Abstract: *The small group of wolves on Isle Royale has been studied for over three decades as a model of the relationship between large carnivores and their prey. During the last ten years the population declined from 50 individuals to as few as 12 individuals. The causes of this decline may be food shortages, disease, or reduced genetic variability. We address the issues of genetic variability and relationships of Isle Royale wolves using allozyme electrophoresis, mtDNA restriction-site analysis, and multilocus hypervariable minisatellite DNA analysis (genetic fingerprinting). Our results indicate that approximately 50% of the allozyme heterozygosity has been lost in the island population, a decline similar to that expected if no immigration had occurred from the mainland. The genetic fingerprinting data indicate that the seven sampled Isle Royale wolves are as similar as captive populations of siblings. Surprisingly, the Isle Royale*

Resumen: *El pequeño grupo de lobos de la Isla Royale ha sido estudiado por más de tres décadas como un modelo de las relaciones entre grandes carnívoros y su presa. Durante los últimos diez años la población disminuyó de 50 individuos a tan sólo 12 individuos. La causa de esta disminución puede ser debida a la escasez de comida, a las enfermedades o a la reducida variabilidad genética. Nosotros tratamos el aspecto de la variabilidad genética y las relaciones con los lobos de la Isla Royale utilizando la electroforésis con aloenzimas, el análisis restringido a mtDNA y el análisis de hipervariable multilocus minisatelite DNA (huellas genéticas). Nuestros resultados indican que aproximadamente el 50% de la heterozigosis de la aloenzima ha sido perdida en la población de la isla, esta es una disminución similar a la que se esperaria si no hubiese habido inmigración de la tierra firme. Los datos de las huellas genéticas indican que los siete lobos muestreados en la Isla Royale son tan parecidos entresí como las poblaciones de hermanos en cautiverio. Sorprendentemente los lobos de la Isla Royale tienen un genotipo mtDNA que es muy raro en tierra firme habiendo sido encontrado en sólamente uno de*

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Paper submitted May 7, 1990; revised manuscript accepted October 10, 1990. See table of contents for order of author's names for citation purposes.

wolves have an mtDNA genotype that is very rare on the mainland, being found in only one of 144 mainland wolves. This suggests that the remaining Isle Royale wolves are probably derived from a single female founder.

los 151 lobos de tierra firme. Esto sugiere que es probabla que los lobos que quedan en la Isla Royale se hayan derivado de una sola hembra fundadora.

Introduction

Gray wolves (*Canis lupus*) were probably established on Isle Royale in Lake Superior about 1949 (Mech 1966). A single gray wolf pair likely founded the island population, which has numbered as many as 50 individuals in the past (Mech 1966; Peterson 1977; Peterson & Page 1988). The relationship between gray wolves and their principal prey, the moose (*Alces alces*), has been the subject of three decades of research continuing to the present (Mech 1966; Jordan et al. 1967; Wolfe & Allen 1973; Peterson 1977; Peterson & Page 1988). The simple island system has provided an unparalleled natural experiment on the relationship between large predators and their prey. Isle Royale was designated an International Biosphere Reserve in 1980 in recognition of its scientific value and its unique fauna and flora.

Wolf numbers on Isle Royale have dropped from 50 individuals in 1980 to approximately 14 in March 1990 (Peterson & Page 1988; Peterson, unpublished data). High mortality during this period was accompanied by a steady decline in the number of reproducing females as indicated by sightings of young at least nine months old. Three possible explanations have been suggested for the reproductive failure and population decline of Isle Royale wolves (Peterson & Krumenaker 1989). First, the Isle Royale wolf population has long been considered to be regulated by food levels (Mech 1966; Peterson 1977; Peterson & Page 1988). Thus, the decline might reflect a decrease in prey availability. Second, island populations may be greatly affected by disease because of their small size and lack of genetic variability, which make recovery from epizootics less likely (Gilpin & Soulé 1986; O'Brien & Evermann 1988). The recent appearance of canine parvovirus in Minnesota and Michigan dogs coincides with the decline of Isle Royale wolves, suggesting that this virus was a possible source of mortality (Mech et al. 1986; Peterson & Page 1988). Finally, because genetic exchange with mainland wolves is limited (Mech 1966; Peterson & Page 1988), and assuming that the effective population size of Isle Royale wolves is no greater than the observed two or three breeding pairs and that five to seven generations have passed since wolves were established on the island, approximately 39–65% of genetic heterozygosity has probably been lost (Crow & Kimura 1970; Barton & Charlesworth 1984). Such extreme losses in heterozygosity may have negative effects on viability and juvenile survival (Ralls & Ballou 1983; Ralls et al. 1988; O'Brien & Evermann 1988).

In this study, we compare genetic variability in Isle Royale wolves to that of mainland populations and evaluate the role genetic variability may have in the population decline. We use three genetic techniques: mitochondrial DNA (mtDNA) restriction-site analysis, allozyme electrophoresis, and analysis of hypervariable minisatellite DNA. These techniques provide estimates of genetic variability and may sometimes be useful tools for reconstructing the relationships of recently isolated populations (Avise et al. 1987; Gilbert et al. 1990).

Materials and Methods

Sampling Design

Wolves were live-captured at both ends of Isle Royale and immobilized with a ketamine/xylazine combination (Kreeger et al. 1987). A 10 cc blood sample was collected for disease, nutrition, and genetic analyses, and each individual was radio-collared and then released. Spring-fall trapping efforts in 1988 and 1989 resulted in capture and blood-sampling of 7 of the 12 remaining wolves in March 1989. No mortality or debilitating injuries resulted from these trapping activities. In addition, 144 blood and organ samples of mainland wolves from Manitoba, Minnesota, Ontario, and Quebec were obtained through other gray wolf research or management programs (Fig. 1, Table 1). Blood samples of wolves from captive colonies at Dalhousie University, Halifax, Nova Scotia, and the Julian Science Center, Julian, California, were obtained as a reference for the level of genetic similarity expected among siblings (Table 1). The Dalhousie sample consists of five descendants from a single mated pair: one F1 individual and two sib pairs with different F1 parents. The average inbreeding coefficient, F, of these animals is 0.275. The Julian Science Center sample consists of 15 siblings from multiple litters of a single parental pair ($F = 0.250$).

Protein Electrophoresis

Protein electrophoresis was done on a subset of the total available wolf sample (Table 2). Red cells isolated from whole blood of wolves were sonicated in buffer and subjected to three freeze-thaw cycles (Wayne & O'Brien 1987). After a high-speed spin, 10 µl of the

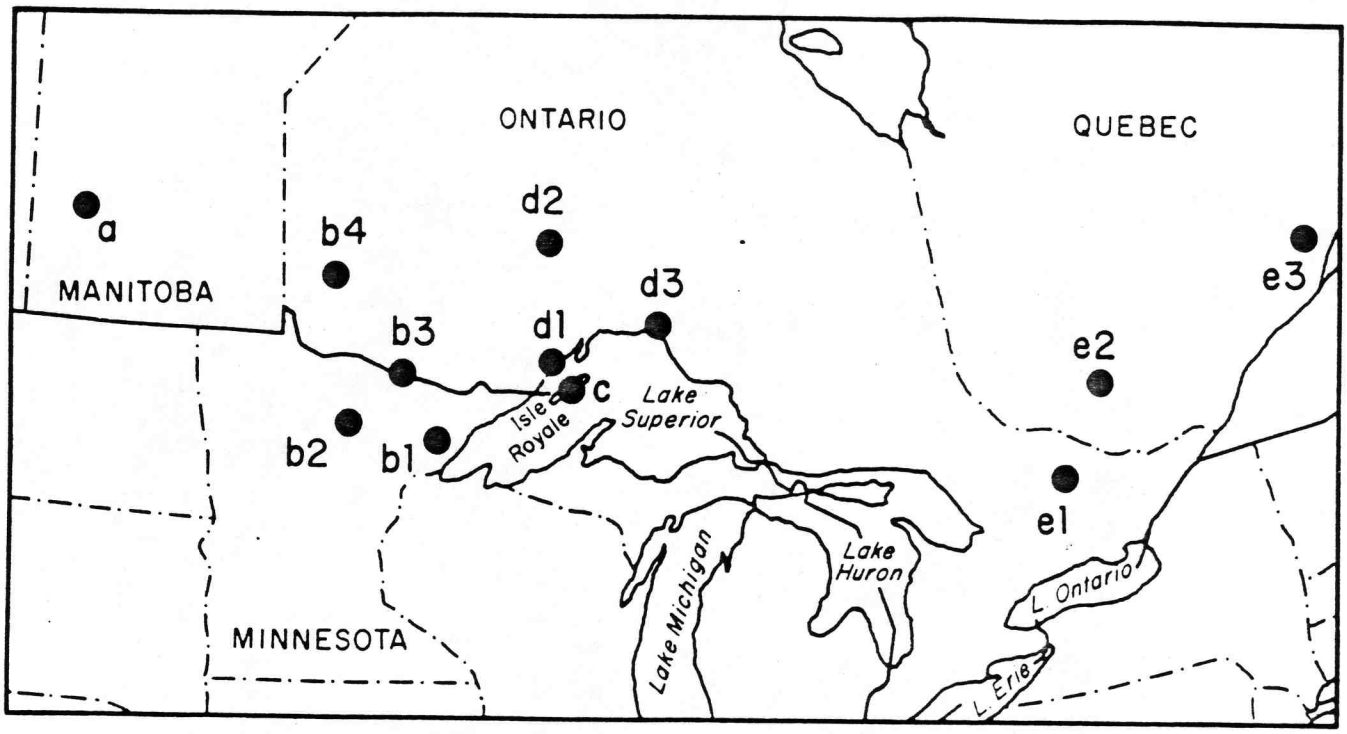

Figure 1. Map of localities in the United States and Canada where blood or organ samples of gray wolves, Canis lupus, *were obtained. For locality identities, see Table 1.*

supernatant was used in protein electrophoresis and staining of 25 soluble blood proteins: ACP-1, ADA, AK-1, CA-1, CAT, DIA-1, DIA-4, ES-1, ES-2, G6PD, GPI, GSR, HB, HEX-A, IDH-1, LDH-B, MDH-2, MPI, NP, PEP-B, PEP-C, PEP-D, SOD1, TF, and XDH (George 1986; Wayne & O'Brien 1987). Gels were scored immediately after stain development and frozen for later comparisons.

Estimates of genetic polymorphism and heterozygosity of island and mainland wolves were calculated from the allozyme data (Nei 1987, 1978). Deviations from Hardy-Weinberg equilibrium were tested using the Chi-square test (Hartl 1980). The standardized variance in allele frequencies among populations, F_{ST} (Wright 1965), was calculated for the singl and multiple allele case using modifications described in Nei (1977) and Nei and Chesser (1983). Weir and Cockerham's (1984)

Table 1. Locality, sample size (n), analyses, and contributor of gray wolf samples (see Fig. 1). GF = genetic fingerprinting.

Locality	*n*	*Analyses*	*Contributor*
a. Riding Mountain NP, Manitoba	2	allozymes, mtDNA	L. Carbyn
b. Minnesota & Northwest Ontario	110		
1. Northeast Minnesota	58	allozymes, mtDNA, GF	D. Mech
2. Northern Minnesota	18	allozymes, mtDNA	B. Paul
3. Voyageurs NP, Minnesota	17	allozymes, mtDNA	P. Gogan
4. Northwest Ontario	17	mtDNA	
c. Isle Royale NP, Michigan	7	allozymes, mtDNA, GF	R. Peterson
d. Central Ontario	16		
1. Thunder Bay	8	mtDNA	R. Peterson
2. Armstrong Station	4	mtDNA	R. Peterson
3. Nipigon	4	mtDNA	R. Peterson
e. Quebec & East Ontario	16		
1. Algonquin Provincial Park	5	mtDNA	G. Forbes
2. Southern Quebec	7	mtDNA	F. Potvin
3. Laurentides Provincial Park	4	mtDNA	F. Potvin
f. Captive colonies	20		
1. Dalhousie University, N.S.	5	GF	J. Ryan
2. Julian Science Center, CA.	15	GF	P. Kenis
Total	171		

Table 2. Allele frequencies by locality, F_{st}, theta (θ), Chi-square, and probability for frequency differences for island and pooled mainland samples. Observed (Het-OB) and expected Hardy-Weinberg (Het-EX) heterozygosity and their standard errors (SE) averaged over 25 loci are provided. Sample size given in parenthesis below locality labels.

		Island	*Mainland*					
Locus	*Allele*	*c1 (7)*	*b1 (16)*	*b2 (6)*	*b3 (11)*	*Pooled (33)*	*F_{st} (θ)*	*Chi-square (probability)*
DIA-1	A	1.00	0.34	0.42	0.32	0.35	0.48	19.7
	B	0.00	0.66	0.58	0.68	0.65	(0.50)	(0.00)
MDH-2	A	0.00	0.32	0.50	0.41	0.38	0.24	7.8
	B	1.00	0.68	0.50	0.59	0.62	(0.23)	(0.01)
MPI-1	A	0.00	0.12	0.17	0.18	0.15	0.08	2.4
	B	1.00	0.88	0.83	0.82	0.85	(0.06)	(0.12)
NP	A	0.71	0.26	0.50	0.46	0.37	0.12	5.7
	B	0.29	0.74	0.50	0.54	0.63	(0.16)	(0.02)
PEP-B	A	0.50	0.44	0.42	0.50	0.46	0.00	0.9
	B	0.50	0.56	0.58	0.50	0.54	(0.00)	(0.76)
Mean F_{ST}		—	—	—	—	0.061	0.19 (0.29)	
Het-OB (SE)		0.040 (0.020)	0.053 (0.023)	0.080 (0.039)	0.062 (0.027)	(0.026)		
Het-EX (SE)		0.039 (0.018)	0.082 (0.035)	0.098 (0.041)	0.093 (0.039)	0.087 (0.036)		

approximation of F_{ST}, known as theta, was also calculated using the computer program developed by Leslie (1989). Statistical significance of differences in allele frequency values was evaluated with a modified Chi-square test (Workman & Niswander 1970). The standard error of mean heterozygosity values for the Isle Royale wolves was corrected for finite sample size using the formula $[(N\text{-}n/N\text{-}1)^{*}se^{2}]^{0.5}$ where N is population size, n is sample size, and se is the standard error (Wannacott & Wannacott 1969). Statistical analyses were done with the program BIOSYS-1 (Swofford & Selander 1981).

Parametric tests may not be robust for heterozygosity data if less than forty loci are used and heterozygosity values are low (Archie 1985). Therefore, we used a computer simulation to determine if the heterozygosity was significantly lower in the island (7 individuals) than in the mainland (33 individuals) population. The simulation involved iterative sampling of 7 individuals at random from the combined mainland and island sample 10,000 times. The heterozygosity of the 7 sampled individuals and the difference between this heterozygosity value and that of the remaining 33 individuals were calculated for each iteration. The number of samples of 7 that had heterozygosity values equal to or less than that observed in the actual island sample served as a measure of significance.

Mitochondrial DNA

Total genomic DNA was isolated from white blood cells separated from whole blood or from organ samples (see Wayne et al. 1989). The DNA solution was ethanol-precipitated and resuspended in a volume of TE buffer to a final concentration of approximately 1–2 μg/μl. Two to 3 μg of DNA were digested separately with the following 21 restriction endonucleases: *Acc*I, *Apa*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Bst*UI, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hba*I, *Hin*cII, *Hin*dIII, *Nco*I, *Sca*I, *Sst*I, *Stu*I, *Xba*I, and *Xmn*I. After electrophoresis through 1% agarose gels, DNA was transferred to Nylon membranes and probed with cloned radio-labeled mtDNA originally isolated from the domestic dog. After autoradiography, fragments were sized according to the molecular standard that was run on each gel (Lamda DNA, *Hin*DIII cut, mixed with ϕX174 DNA, *Hae*III cut). Fragment sizes were summed for each individual to determine if the entire mtDNA genome was represented (approximately 16.8 kb) and to check for deletions or insertions and heteroplasmy (cf. Densmore et al. 1985; Boursot et al. 1987). Fragment identity was assessed on the basis of comigration of fragments separated in the same gel.

The restriction-fragment patterns for each individual for the 21 restriction enzymes were used to define composite mtDNA genotypes (Lansman et al. 1981, 1983; Bermingham & Avise 1986; Wayne et al. 1990). Restriction-site differences were readily estimated from fragment patterns because all genotypes differed by the inferred loss or gain of only one or two restriction sites for each restriction enzyme. A per-genotype presence-absence matrix of restriction sites was used to calculate a maximum parsimony tree relating mtDNA genotypes with the branch-and-bound option in the PAUP program, version 2.4, by David Swofford (1985). The percent nucleotide sequence divergence was calculated between genotypes using the "site" method (Nei & Li 1979).

Multilocus Analysis

Genomic DNA from canids was digested with the restriction endonuclease *Hin*fI and probed with hypervariable minisatellite DNA, clone 33.6, as outlined by Jeffreys et al. (1985) and Gilbert et al. (1990). Several exposures of autoradiographs were made, varying from 1 to 10 days, to accurately score bands of differing intensity. All bands in the molecular weight range of 1.0 to 12.0 kb were scored; those showing a similar molecular weight and intensity were considered to be identical (Fig. 2). Only individuals on the same gel were compared. The difference value (D) between the restriction-fragment patterns of two individuals was calculated as the number of fragments that differed between the two individuals divided by the total number of fragments. The average percent difference (APD) is the average of all D values for each population times 100 (Gilbert et al. 1990). Because of the nonindependence of pairwise D values, an unbiased estimation of the standard error may be defined as $[2\bar{D}(1 - \bar{D})(1 + \bar{D})/n(3 + \bar{D})]^{0.5}$ where n = mean number of restriction fragments per individual and $\bar{D}$ is the mean of all pairwise D values (Lynch 1990).

Results

Allozyme Variability

Five (20%) of the 25 loci examined were polymorphic in mainland wolves whereas only two of these loci (8%) were variable in the sample of Isle Royale wolves (Table 2). For three loci, DIA-1, MDH-2, and NP, allele frequency differences between mainland and island wolves were significant (Table 2, Chi-square test, $P < 0.05$). In contrast, among three mainland populations that were surveyed no significant differences in allele frequencies were detected. Thus, it appears that both allele frequency changes and probable allele loss have occurred in the island population. However, the five to seven remaining Isle Royale wolves need to be surveyed to determine with certainty the absolute number of alleles lost.

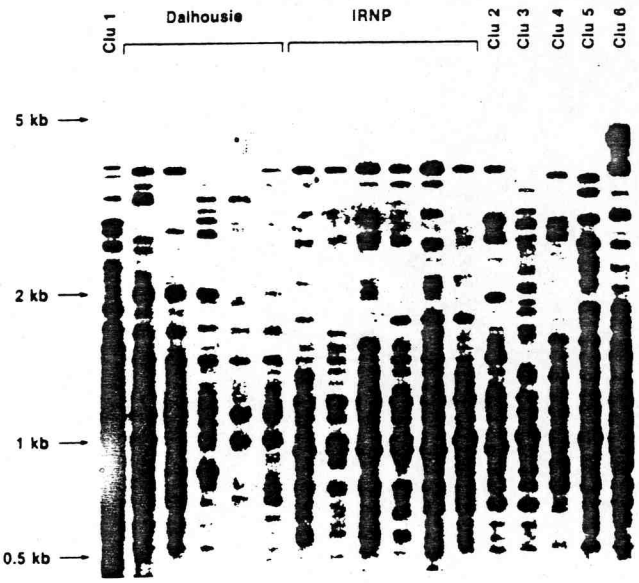

Figure 2. Example autoradiogram of genomic DNA from mainland (Clu1-6), Isle Royale (IRNP), and Dalhousie wolves digested with Hinf*I and probed with Jeffreys' 33.6 clone.*

As indicated by a Chi-square test, genotype frequencies were not in Hardy-Weinberg equilibrium 2 out of 17 times for which a population is polymorphic for an individual locus ($p < 0.05$). These deviations were due to heterozygote deficiencies at two loci, MDH and NP, in wolves at locality b1 (Fig. 1). This was consistent with the Wahlund effect and thus may reflect population subdivision on a microgeographic scale. Such subdivision may indicate a lack of gene flow among closely situated wolf packs.

The average F_{ST} and theta values for comparisons of island wolves and the pooled sample of mainland wolves are near 0.20 and 0.30, respectively (Table 2). Thus, approximately 20–30% of the variance in allele frequency is due to between-population differences. This suggests that given the short period of isolation, founder effect and drift have resulted in significant differentiation between mainland and island populations. This result also indicates that levels of gene flow are low.

The unbiased estimate of allozyme heterozygosity in Isle Royale wolves, 0.039, was approximately half that of the mainland wolves (Table 2). This value or lower was found in only 3 of 10,000 random samplings of seven individuals from the total sample population ($P < 0.001$). In no sample iteration was the difference in heterozygosity values greater or equal to that observed in the actual sample. This occured so infrequently because the seven Isle Royale wolves were fixed for the same allele at three loci, DIA-1, MDH-2, and MPI-1 (Table 2). Such coincident monomorphism is extremely rare in our sample of mainland wolves. Hence, we regard the heterozygosity of mainland and island wolves as significantly different.

mtDNA Variability

Nine mtDNA genotypes were defined by restriction-site analysis of island and mainland wolves (Table 3). Only one genotype (W8) is found in Isle Royale wolves. Significantly, this genotype is found only in one wolf in a total sample of 144 mainland wolves and is from locality d3, approximately 100 km northeast of Isle Royale (Fig. 1, Table 3). Among Minnesota and Northwest Ontario wolves (b1–b4), four genotypes are found; three are

Table 3. Frequencies and samples size in parenthesis of mtDNA genotypes of wolves at localities in Manitoba, Minnesota, Ontario, and Quebec (see Fig. 1). Locality c is Isle Royale[a].

	mtDNA genotypes								
Locality	*W1*	*W2*	*W4*	*W7*	*W8*	*W9*	*W10*	*W11*	*W12*
a	—	1.00 (2)	—	—	—	—	—	—	—
b1–b4	0.34 (37)	—	0.01 (1)	0.32 (35)	—	0.34 (37)	—	—	—
c	—	—	—	—	1.00 (7)	—	—	—	—
d1–d3	—	—	0.25 (4)	0.25 (4)	0.06 (1)	0.38 (6)	0.06 (1)	—	—
e1–e3	—	—	—	—	—	—	0.44 (7)	0.13 (2)	0.44 (7)
Total	0.25 (37)	0.01 (2)	0.03 (5)	0.26 (39)	0.05 (8)	0.28 (43)	0.05 (8)	0.01 (2)	0.05 (7)

[a] *Mitochondrial DNA genotypes are numbered as defined in Lehman et al. In press.*

relatively abundant (W1, W7, and W9) and one is rare (W4). In east Ontario and Quebec wolves (e1–e3), three mtDNA genotypes exist (W10, W11, and W12) that are not found in Minnesota or Northwest Ontario, suggesting geographic partitioning. Similarly, the mtDNA genotype found in Manitoba (W2) is not present in wolves at the other localities.

Sequence divergence data indicate that the Isle Royale wolf genotype is closely related to several genotypes found on the mainland (Fig. 3, Table 4). The Isle Royale wolf genotype (W8) is one restriction site or approximately 0.1% different in DNA sequence from the mtDNA genotype W7 found in wolves in Minnesota and Ontario. It is less than 0.7% different in mtDNA sequence from mtDNA genotypes W9, W10, W11, and W12 (Table 4). However, the Isle Royale genotype is dramatically different, with approximately 3.5% sequence divergence, from the remaining wolf genotypes found on the mainland (W1, W2, and W4). Based on a study of coyote mtDNA genotyp s (Lehman et al., in press), we concluded that this difference reflects interspecific hybridization between wolves and coyotes, so we included samples of 15 coyotes from Ontario, Minnesota, and Michigan in the restriction site analysis. The maximum parsimony tree in Figure 3 clearly suggests that wolf mtDNA genotypes W7 through W12, including the Isle Royale wolf genotype (W8), are closely related to, or indistinguishable from, mtDNA genotypes found in coyotes. Thus, in Minnesotan and Canadian wolves there are two distinct genotype groups, one including three mtDNA genotypes that are true wolf genotypes (W1, W2, and W4), and the other including genotypes derived from hybridization with coyotes such as the Isle Royale genotype (W8) and all wolf genotypes found in Quebec (W10, W11, and W12).

Multilocus Analysis

The restriction-fragment profiles and APD values differ substantially between related and unrelated individuals

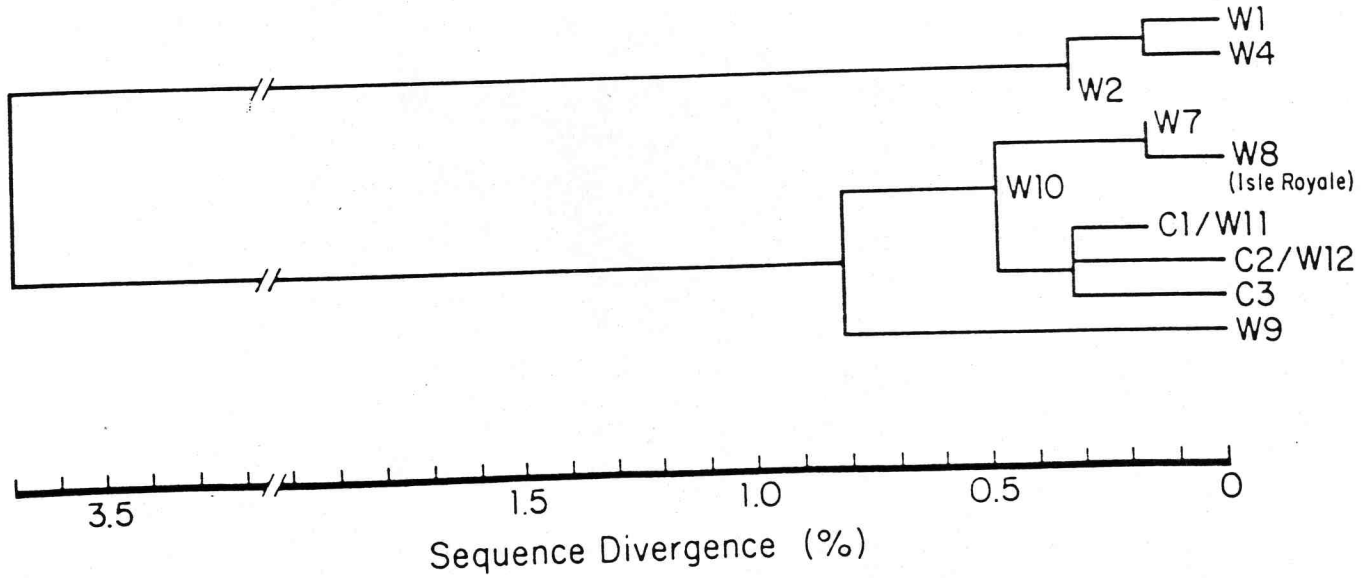

Figure 3. A most parsimonious tree of gray wolf and coyote mtDNA genotypes. The tree is rooted at the midpoint of the longest path connecting any pair of taxa. Tree length = 44, consistency index = 0.98. Codes: W1–W4, pure wolf genotypes; W7–W12, wolf genotypes derived from coyotes (W8 is the Isle Royale genotype); C1–C3, coyote genotypes found in 15 coyotes from Minnesota, Ontario, and Michigan (see Table 4).

(Fig. 2, Table 5). Individuals known to be as closely related as siblings from the captive colonies at Dalhousie University and the Julian Science Center have APD values of 31.7% ± 8.5% and 31.9% ± 9.6%, respectively. By contrast, presumed unrelated individuals have an APD value of 68.5% ± 7.8%. The range of difference values for unrelated and related individuals does not overlap. The Isle Royale wolves have an APD of 28.5% ± 6.7% and a range of difference values that are similar to those of wolves related as siblings. These results may be viewed graphically in a cluster analysis in which individuals are grouped according to their pairwise difference values (Fig. 4). The Isle Royale wolves are grouped in a tight cluster just as are wolves in the Dalhousie colony. Thus, with respect to hypervariable minisatellite loci, the Isle Royale wolves appear genetically as close as siblings.

Discussion

Genetic Variability of Isle Royale Wolves

In island populations, genetic variability is decreased through founder effect and genetic drift (Wright 1969; Allendorf 1986). The loss due to the former reflects the number of founding individuals and their genetic composition. The decrease due to drift reflects the intrinsic growth rate and the harmonic mean of the effective population size over time (Wright 1969; Nei et al. 1975). An approximate expression for the expected loss in genetic heterozygosity is given by $H_{n+1} = (1 - \frac{1}{2}N_0)(1 - \frac{1}{2}N_1)\ldots(1 - \frac{1}{2}N_n)H_0$ where N equals the number of effective individuals at generation n and H_0 is the heterozygosity of the ancestral population (Crow & Kimura 1970; Barton & Charlesworth 1984). Thus, given five to seven generations and two or three breeding pairs per generation, the expected loss in heterozygosity ranges from 39–65%. However, the stochastic variance of the expected loss may be large (Lacy 1987). The actual loss in heterozygosity of the island population relative to wolves on the mainland, as measured by allozyme electrophoresis, is approximately 50%. Thus, Isle Royale wolves have retained a significant proportion of the variability found among mainland wolves. The amount they have lost is consistent with that expected given complete isolation and a small effective population size.

The expected decrease in APD values with inbreeding in the island population is difficult to determine without study of APD values of captive wolves under different systems of close breeding. Recent study of such populations in chickens suggests a nonlinear relationship between band-sharing and the inbreeding coefficient, F (Kuhnlein et al. 1990). The observed decline in APD for Isle Royale wolves is 58% relative to the APD of mainland wolves (Table 5). This decline is only slightly greater than that of sibling wolves in the Julian Science Center (53%) or closely related wolves in the Dalhousie colony (54%) and suggests only a few generations of inbreeding have taken place following the initial founding.

Several studies have suggested a link between levels of heterozygosity and fitness traits, but the specific correspondence between a given loss in heterozygosity and a drop in fitness probably depends on the species-specific ability to withstand inbreeding (Shields 1982; Templeton & Reed 1984; Templeton 1986; Ralls et al. 1988). The coefficient of inbreeding increases at a rate given by delta F = $\frac{1}{2}N$ where N is the effective population size (Falconer 1981). Assuming two to three reproductive pairs in the island population, delta F is approximately 8–13% per generation. Data from domestic mammals show that traits related to fitness such as litter size may decrease 5–7% with a 10% increase in F (Falconer 1981). Similarly, the average increase in juvenile mortality caused by parent-offspring or full-sib mating in zoo mammals was 33% (Ralls et al. 1988). Thus, inbreeding effects are consistent with the observed small litters and low number of pups that survive to 9 months of age (Peterson & Page 1988).

However, recent behavioral observations of young adult Isle Royale wolves suggest that they may fail to pair-bond successfully (Peterson & Page 1988; Peterson, unpublished data). Their failure to pair-bond and reproduce may indicate that they recognize each other as siblings and are exhibiting incest-avoidance behavior. A speculative scenario is that initially, after several litters were born to the founding pair, breeding pairs were established through the pairing of their offspring. Such pairing may have been facilitated by the mating of offspring from litters separated by several years such that two potential mates might not recognize each other as siblings. Subsequently, the population bottleneck of 14 wolves in 1982 may have resulted in the present population descending from a single or several closely spaced litters of one mated pair (Peterson & Page 1988). Thus, they may recognize each other as siblings and fail to interbreed due to premating behavioral mechanisms.

Relationship of Isle Royale Wolves to Mainland Wolves

Mitochondrial DNA restriction-site analysis is useful for documenting patterns of microgeographic differentiation (Moritz et al. 1987; Avise et al. 1987). The mtDNA sequence of mammals evolves rapidly, five to ten times faster than the average nuclear gene (Brown et al. 1979), such that populations often differ in the predominance of mtDNA genotypes (geographic partitioning). Genotypes from different localities can often be connected in a network reflecting migration patterns among populations (e.g., Lansman et al. 1983; Avise et

Table 4. Percent sequence divergence (above diagonal) and fraction of shared restriction sites (below diagonal) between wolf and coyote mtDNA genotypes. Wolves, W1–W12, *Canis lupus;* and coyotes, C1–C3, *Canis latrans.* Asterisk indicates wolf mtDNA genotypes of coyote origin. Values in bold indicate sequence or restriction-site differences between coyote or coyote-related wolf mtDNA genotypes. W8 is the Isle Royale wolf genotype.

	W1	*W2*	*W4*	*W7**	*W8**	*W9**	*W10**	*W11**	*W12**	*C3*
W1	—	0.2	0.2	3.4	3.6	3.6	3.2	3.3	3.5	3.5
W2	0.987	—	0.2	3.1	3.3	3.3	2.9	3.1	3.2	3.2
W4	0.987	0.987	—	3.4	3.6	3.6	3.2	3.3	3.5	3.5
W7*	0.816	0.829	0.816	—	**0.1**	**0.9**	**0.2**	**0.3**	**0.4**	**0.4**
W8*	0.808	0.821	0.808	**0.993**	—	**0.1**	**0.3**	**0.4**	**0.6**	**0.6**
W9*	0.808	0.822	0.808	**0.972**	**0.935**	—	**0.7**	**0.6**	**0.7**	**0.7**
W10*	0.827	0.840	0.827	**0.986**	**0.979**	**0.957**	—	**0.1**	**0.2**	**0.2**
W11*/C1	0.819	0.832	0.819	**0.979**	**0.972**	**0.964**	**0.993**	—	**0.1**	**0.1**
W12*/C2	0.813	0.827	0.813	**0.972**	**0.965**	**0.957**	**0.986**	**0.993**	—	**0.2**
C3	0.813	0.827	0.813	**0.972**	**0.965**	**0.957**	**0.986**	**0.993**	**0.986**	—

al. 1987). However, relatively few studies have dealt with highly mobile vertebrates, such as wolves, which potentially disperse over long distances in search of mates (Mech 1987). Thus, similarity in genotype frequencies at localities in Minnesota and Northwest Ontario might be expected given the dispersal capabilities of wolves. However, the appearance of unique, coyote-derived genotypes in east Ontario and Quebec wolves may indicate that dispersal between Minnesota and Quebec is limited and that hybridization between wolves and Quebec coyotes (with unique genotypes) may be occurring (Lehman et al., in press).

The Isle Royale wolf genotype appears to be very rare in mainland wolves (1/144, Table 3). Thus, the probability of two unrelated females with this genotype founding the Isle Royale population is small, supporting the contention that the sampled wolves descended from a single founding female. However, the Isle Royale genotype may be more abundant in areas outside of Minnesota and Ontario. All wolf mtDNA genotypes sampled from Quebec are derived from coyotes. This reflects multiple hybridization events, and consequently the Isle Royale genotype could have originated in coyotes or wolves to the east of Ontario. Wolves found to the west and north of Minnesota and Ontario all have pure wolf mtDNA genotypes, making them an unlikely source of the coyote-derived mtDNA genotype found in wolves from Isle Royale (Lehman et al., in press). Consequently, the presence of a rare genotype in Isle Royale wolves may reflect long-distance dispersal of wolves from areas to the east of Isle Royale. We are currently expanding our sample to test this hypothesis.

Conservation Implications

Conservation plans often focus on the protection of endangered populations that are morphologically or genetically distinct. The Isle Royale wolves appeared to be a population that would not be distinct from most mainland wolves given that they have been isolated for only 40 years. However, the Isle Royale wolves have a mtDNA genotype that is extremely rare on the mainland. This might be reason for conservation of Isle Royale wolves as a unique genetic entity. However, such concerns need to be placed in the perspective of variation within the species and additional morphological and genetic criteria. The single restriction-site difference between the Isle Royale wolf genotype and other genotypes such as W7 on the mainland is small relative to a maximum of 16 observed restriction-site differences separating coyote genotypes (Lehman et al., in press). Moreover, no allozyme markers (Table 2) or morphological differences distinguishing mainland and Isle Royale wolves are evident (Peterson 1977 and unpublished data). Therefore, the limited morphological and genetic distinctiveness of Isle Royale wolves does

Table 5. Sample size (N), average percent difference (APD), range, standard error (SE) with unbiased estimate in parenthesis, mean number of restriction fragments with standard deviation in parenthesis, and proportion of fixed restriction fragments.

	N	*APD*	*Range*	*SE*	*Mean number of fragments*	*Proportion fixed*
c. Isle Royale	7	28.5	18.9–40.5	6.7 (9.1)	19.1 (1.0)	0.42
f1. Dalhousie	5	31.7	17.6–42.9	8.5 (8.8)	17.4 (0.7)	0.35
f2. Julian Science	15	31.9	11.1–46.7	9.6 (10.2)	16.5 (1.6)	0.30
b1–4 (Mainland)	6	68.5	51.2–82.4	7.8 (10.1)	19.5 (1.9)	0.15

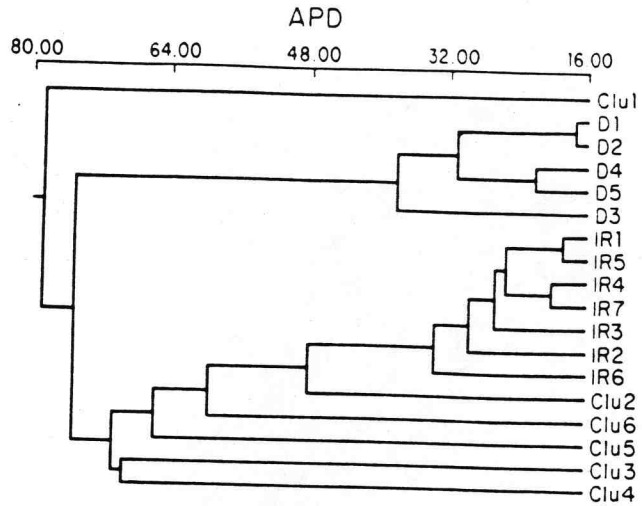

Figure 4. UPGMA tree based on average percent difference (APD) values from the genetic fingerprinting analysis of mainland, island, and captive wolves (Table 5). Tree generated using the Numerical Taxonomy System (Applied Biostatistics Inc., Setauket, New York). Codes: D1–D5, wolves from Dalhousie University; IR1–IR7, Isle Royale wolves; Clu1–Clu6, mainland wolves chosen at random from Minnesota.

not support conservation of Isle Royale wolves as a separate entity.

A second consideration concerns reintroduction of mainland wolves to Isle Royale given the possible extinction of the resident population. The Isle Royale wolf population has scientific value as a model of persistence and genetic change in small populations, and as a model for predator-prey relationships. Consequently, reintroduction of wolves from the mainland may be desirable, but which wolf mtDNA genotypes should be reintroduced? Introduction of wolves with coyote genotypes necessarily involves wolves whose ancestors have hybridized with coyotes. Alternatively, introduction of wolves with wolf mtDNA may compromise the present resident population because they have a coyote-derived mtDNA genotype. However, given that maintaining the genetic integrity of the island population is not critical, individuals should be chosen with unique genetic markers to allow monitoring of reproduction in the population over time. For example, females with different mtDNA genotypes can be introduced into the resident population. Offspring survival could then be effectively monitored with mtDNA restriction-site analysis and genetic fingerprinting.

Acknowledgments

We thank the biologists listed in Table 1, as well as M. Nelson, J. Burch, and numerous others for sending specimens. Funding was provided by the U.S. National Park Service to R.K.W. and R.O.P., by the National Science Foundation (BSR-8716095) to R.O.P., and by the U.S. Fish and Wildlife Service and the U.S.D.A. North Central Forest Experiment Station. J. Archie, J. Hatfield, T. Fuller, F. Hertel, R. Nowak, S. O'Brien, P. Smouse, B. Van Valkenburgh, and two anonymous reviewers provided comments that greatly improved the manuscript.

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