Genetic Structure and Migration in Native and Reintroduced Rocky Mountain Wolf Populations

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Abstract: *Gray wolf (*Canis lupus*) recovery in the Rocky Mountains of the U.S. is proceeding by both natural recolonization and managed reintroduction. We used DNA microsatellite analysis of wolves transplanted from Canada to two reintroduction sites in the U.S. to study population structure in native and reintroduced wolf populations. Gene flow due to migration between regions in Canada is substantial, and all three recovery populations in the U.S. had high genetic variation. The reintroduced founders were moderately genetically divergent from the naturally colonizing U.S. population. These findings corroborate that the reintroduction more than meets generally accepted genetic guidelines. Maintaining this variation, however, will depend on ample reproduction in the first few generations. In the long term genetic variation will best be retained if migration occurs among the recolonizing and the two transplanted populations. Evidence from field observation and genetic studies shows extensive dispersal by wolves, and we conclude that exchange among these groups due to natural dispersal is likely if public tolerance and legal protection are adequate outside lands designated for wolf recovery.*

Estructura Genética y Migración de Poblaciones Nativas y Reintroducidas del Lobo de las Montañas Rocallosas

Resumen: *La recuperacion del lobo gris (*Canis lupus*) en las montañas Rocallosas de los Estados Unidos ha procedido tanto de la recolonización natural, como de la reintroducción controlada. Para estudiar la estructura poblacional de lobos nativos y reintroducidos, utilizamos análisis de microsatélites de ADN de lobos transplantados de Canada hacia dos sitios de reintroducción en los Estados Unidos. El flujo de genes debido a la migración entre regiones del Canada es sustancial y las tres poblaciones en recuperación de Estados Unidos tuvieron una alta variación genética. Los fundadores de las reintroducciones fueron moderadamente divergentes de las poblaciones colonizadoras naturales desde el punto de vista genético. Estos resultados corroboran que la reintroducción concuerda mas que bien con los lineamientos genéticos generalmente aceptados. Sin embargo, mantener esta variación dependerá en gran medida de la reproducción de las primeras generaciones. En un largo plazo, la variación genética será retenida al máximo si ocurren migraciones entre las problaciones recolonizadoras y las transplantadas. Evidencias de campo y estudios de genética muestran una dispersión extensiva de los lobos y concluímos que el intercambio entre estos grupos debido a la dispersión natural es posible, siempre y cuando la tolerancia del público y la protección legal sean adecuadas fuera de lás tierras designadas como áreas de recuperación de los lobos.*

Introduction

Wild canid populations worldwide vary in status from secure to fragmented, isolated, hybridized, or locally extinct. Canid conservation genetics has benefitted from the development of DNA microsatellite loci in the domestic dog (Ostrander et al. 1993; Gottelli et al. 1994; Roy et al. 1994; Garcia-Moreno et al. 1996; Forbes & Boyd 1996). The large number and high variability of these DNA markers make genetic studies of wild canids increasingly informative.

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Wolves in the central Rocky Mountains have a history of persecution and tenuous recovery (Gunson 1992; Boyd et al. 1995; we consider the Rocky Mountains in their entirety: the central Rockies span the Canada-U.S. international boundary). Previously we used DNA microsatellites to study wolves that naturally recolonized western Montana from Canada from 1985–1995. High genetic variation in the U.S. wolves indicated that there was not a founding population bottleneck sufficient to diminish genetic variation during colonization (Forbes & Boyd 1996). All evidence from genetic and field data indicated that natural dispersal in wolves was adequate to preclude any concern about inbreeding in the colonizing population. The Montana population has grown to approximately 70 in at least seven breeding packs, occupying a region extending 350 km south of the Canadian border in Montana (Fritts et al. 1995; Fig. 1).

Wolves are endangered in the lower 48 states, but are numerous in large parts of Canada. The area of natural recolonization in Montana is one of three areas designated for wolf recovery in the western U.S. (U.S. Fish and Wildlife Service 1987, 1994). To further the recovery effort, during the winters of 1995 and 1996 wolves were trapped in central Alberta and northern British Columbia and transported to the other two recovery areas in Yellowstone National Park (YNP) and central Idaho, south and southwest of the naturally recolonizing population (Bangs & Fritts 1996; Fritts et al. 1997; Fig. 1).

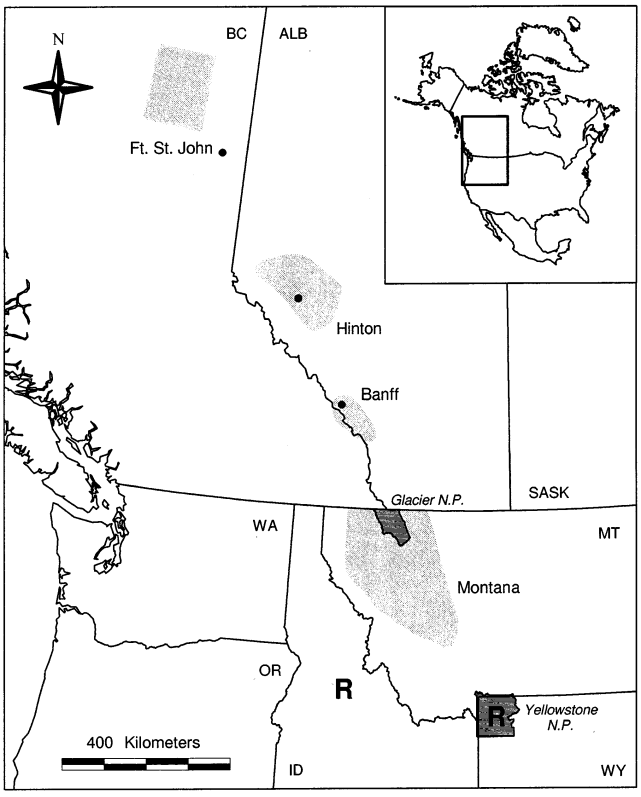

Figure 1. Map of Rocky Mountain wolf range. Shaded areas in Canada indicate origins of the Banff sample, the 1995 Hinton transplants, and the 1996 Fort St. John transplants. The shaded area in Montana indicates the range of the recolonizing population. Indicated areas (R) in Yellowstone National Park and central Idaho are reintroduction sites used in both 1995 and 1996.

We augmented the previous database with genotypes of all the transplanted wolves. The new data add to our knowledge of wolf population structure in Canada and provide a baseline for the initial genetic variation in U.S. wolf reintroduction areas. Our goal is to combine these genetic data with 15 years of field observation in Montana to better understand the genetic and demographic effects of both natural and managed wolf dispersal.

Study Populations and Methods

Montana wolf recovery was monitored from the late 1970s to the present by the University of Montana Wolf Ecology Project (Ream et al. 1991) and the U.S. Fish and Wildlife Service (Fritts et al. 1995). Several animals from each pack were captured, blood sampled, and radiocollared (Boyd et al. 1995). The Banff Wolf Project concurrently monitored wolf populations in Banff, Yoho, and Kootenay National Parks of Canada (Paquet 1993).

The six Rocky Mountain wolf samples differ in population history and sampling structure. The four samples of naturally resident wolves (Fort St. John, Hinton, Banff, Montana) come from a 1350 km range in the northern and central Rockies (Fig. 1). The Fort St. John and Hinton animals are from resident populations where wolves were at times persecuted but never extirpated; Banff wolves were locally extirpated but recovered in the 1980s (Gunson 1983; 1992; Tompa 1983).

The Hinton and Fort St. John wolves were sampled when they were captured for translocation to the U.S. in 1995 and 1996, respectively (Bangs & Fritts 1996; Fritts et al. 1997). In each year approximately half of the wolves were released in YNP and half in central Idaho (Table 1). Thus, each introduced population is a mixture formed from the two Canadian sources. This reintroduction pattern means that population sampling differs among regions. The Fort St. John and Hinton samples were small subsets of large native populations, but these same animals are a complete sample of the reintroduced YNP and Idaho wolves. The Banff and Montana samples fall in between: they are not complete samples, but they do include members of all resident packs known to researchers. Allele frequencies for the Banff and Montana samples were previously reported (Forbes & Boyd 1996). The present dataset (Appendix) includes all the transplanted wolves and adds seven new wolves to the Montana sample. In both reintroduction years nine family

Table 1. Genetic variation at 10 microsatellite loci in Rocky Mountain wolves.[a]

Population	N	A	H_o	H_e
Fort St. John (source)	41.0	4.5	0.588	0.589
Hinton (source)	33.0	4.5	0.579	0.628
Banff	32.0	4.4	0.553	0.581
Montana (recolonized)	66.0	4.1	0.606	0.606
Yellowstone (founders)[b]	31.0	4.7	0.591	0.635
Idaho (founders)[c]	35.0	4.6	0.589	0.636
Total[d]	172.0	5.4	0.587	0.641

[a] *N, mean sample size per locus;* A, *mean number of alleles per locus;* H_o, *observed heterozygosity, and* H_e, *binomial (Hardy-Weinberg) expected heterozygosity (unbiased estimate). Eight wolves sampled at Fort St. John and Hinton were released and not transported to the U.S.*
[b] *Fourteen wolves from Hinton (1995) and 17 from Fort St. John (1996).*
[c] *Fifteen wolves from Hinton (1995) and 20 from Fort St. John (1996).*
[d] *One hundred six wolves from Canada and 66 from Montana.*

groups of wolves were collected. In YNP wolves were held in pens and released as family groups based on their pack membership in Canada, whereas in Idaho they were released immediately after transport (Bangs & Fritts 1996; Fritts et al. 1997; Table 1).

Blood samples were taken from live-trapped wolves (Ream et al. 1991; Boyd et al. 1995), and muscle samples were taken from wolves found dead. Laboratory methods were previously described (Forbes & Boyd 1996). The DNA microsatellite loci were amplified from purified DNA or from Chelex tissue preparations using the polymerase chain reaction (PCR). Ten dinucleotide repeat (AC)n loci characterized in the domestic dog (Ostrander et al. 1993) were chosen from those previously used in wolves (Roy et al. 1994; Forbes & Boyd 1996). Nine loci are the same in these two studies.

Population genetic parameters were calculated using BIOSYS-1 (Swofford & Selander 1989). Heterozygosity differences between samples were tested using a paired *t* test on *H* values at individual loci (Nei 1987; Leberg 1992). We estimated population differentiation using the F_{ST} estimator θ (theta; Cockerham & Weir 1993) calculated by the program GENEPOP (Raymond & Rousset 1995). This program also estimates migration rate (N_em) based on genetic differentiation between subpopulations (Slatkin 1987; Slatkin & Barton 1989). Simulation studies showed that θ is the best choice of differentiation measure for estimating migration when a population is continuously distributed without discrete boundaries between demes (Slatkin & Barton 1989), a model that may be most appropriate for Canadian wolves (Nowak 1983). Tests for correlation between genetic differentiation and geographic distance (Slatkin 1993) were also calculated using programs in GENEPOP (DIST by M. Slatkin; and MANTEL by Raymond & Rousset). The Mantel matrix correlation tests are based on Spearman rank correlations (R_S).

Results and Discussion

Genetic Variation

Levels of genetic variation were high in all samples. Average heterozygosity (H_e) in the Canadian populations ranged from 0.581 to 0.628, and the recolonized Montana population fell within this range (H_e = 0.606; Table 1). No two of these values were significantly different. Allelic diversity (the mean number of alleles per locus, *A*) ranged from 4.4 to 4.5 in Canada and was 4.1 in Montana. These levels of variation are comparable to those in wolves from across Canada and Alaska genotyped at 10 microsatellite loci by Roy et al. (1994). In that study only the sample from the Canadian Northwest Territories had significantly higher heterozygosity and more alleles than any of our six Rocky Mountain samples compared at the same nine loci (data not shown). The reintroduced YNP and Idaho groups are unusual population samples because both groups are nearly equal mixtures of animals from the same two sources (Fort St. John and Hinton). Observed heterozygosity is approximately the same in the source groups and in the mixed transplant groups (all H_o = 0.579–0.591; Table 1). Expected heterozygosity (H_e) is higher in the introduced wolves than in the source populations, but this is expected in the combined groups because of allele frequency differences between the source populations (the Wahlund effect).

Population Structure

Random mating (panmixia) is a proper null hypothesis for population structure. Realistically, however, we would not expect panmixia for most large mammals because they are frequently territorial and dispersal distances are generally limited (Chepko-Sade et al. 1987). The simplest indicator of departure from panmixia is allele frequency differentiation among geographically distant samples. Allele frequencies tested over all 10 loci differed significantly among the four native (non-reintroduced) Canadian and Montana samples in all pairwise tests (all $p < 0.001$ when combined over 10 loci). Significant allele frequency differences are compatible with substantial levels of gene flow, however (Wright 1931; 1969; Allendorf & Phelps 1981), and because of high allelic diversity, microsatellites are especially sensitive indicators of allele frequency differentiation. Significant allele frequency differences alone do not demonstrate biologically important isolation.

F-statistics provide more informative measures of population structure. The most important of these is F_{ST}, the proportion of total variation that is due to differences between subpopulations (if F_{ST} = 1, subpopulations have no alleles in common; if F_{ST} = 0, allele frequencies in all subpopulations are identical). Among the three Canadian populations and among all four native popula-

tions (including Montana colonizers) F_{ST} (Nei 1977) was 0.074. This amount of differentiation is moderate for natural populations of animals in general (Nei 1987; Hartl & Clark 1989), and it agrees closely with other studies of wolves at similar geographic distances. Kennedy et al. (1991) also found an F_{ST} of 0.074 in a group of eight wolf subpopulations from northwestern Canada assayed at five polymorphic allozyme loci. In another study using microsatellites, wolves from five populations sampled throughout North America had a predictably greater differentiation (F_{ST} = 0.168; Roy et al. 1994).

Tests for deviation from binomial expected (Hardy-Weinberg) genotype proportions in the four native populations (Fort St. John, Hinton, Banff, Montana) showed significant deviations only in the Hinton sample. In the Hinton wolves two individual loci had significant heterozygote deficits after correcting for the number of tests, and the randomization test combined over all 10 loci was also significant ($p < 0.01$; data not shown). This may be due to a moderate tendency of individuals to breed in or near their natal home range in this population or to a moderate, undetected dispersal barrier.

We also used Nei's standard genetic distance (*D*; Nei 1978) to measure pairwise population differences. Nei's *D*s among the native groups ranged from 0.093 between Banff and Montana to 0.223 between Fort St. John and Banff (Table 2; Fig. 2). Again, these distances are generally small compared to microsatellite *D*s among wolf populations spread throughout the continent, which ranged from 0.182 to 0.418 (Roy et al. 1994).

We tested for correlation between genetic differentiation and geographic distance between samples. In such tests positive correlations indicate isolation-by-distance, where gene flow between subpopulations results in greater similarity between neighboring sub-populations than between distant ones (Slatkin 1993). For these tests we combined our data with those of Roy et al. (1994), using the nine loci in common between the studies. For the four Rocky Mountain samples alone, genetic differentiation and geographic distance were significantly correlated (R_S = 0.829; $p < 0.05$; one-tailed test; Fig. 2, open circles). The 28 pairwise comparisons among all eight samples also showed positive correlation (R_S = 0.652; $p < 0.05$; Fig. 2, all symbols). This test was significant

Table 2. Pairwise genetic distances among Rocky Mountain wolf populations.*

Population	*1*	*2*	*3*	*4*	*5*	*6*
Fort St. John	—					
Hinton	0.150	—				
Banff	0.223	0.127	—			
Montana	0.162	0.145	0.093	—		
Yellowstone founders	0.023	0.028	0.164	0.133	—	
Idaho founders	0.016	0.037	0.137	0.118	0.005	—

**Unbiased standard genetic distance (Nei 1978).*

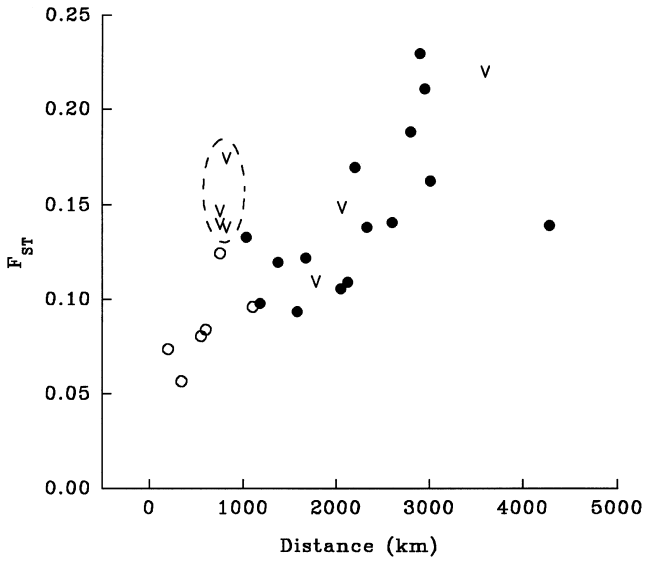

Figure 2. Comparison of genetic differentiation (F_{ST} estimator θ) and geographic distance at nine microsatellite loci among wolf populations. There are 28 pairwise comparisons among eight populations (all symbols): four Rocky Mountain samples from the present study and four more distantly spaced populations (Vancouver Island, Kenai Peninsula, Northwest Territories, and Quebec; data from Roy et al. 1994). The open circles are the comparisons among the four Rocky Mountain samples. The "V" symbols are the comparisons with the Vancouver Island sample. The dashed line surrounds the points comparing Vancouver Island and its four nearest neighbors, which are the four Rocky Mountain samples.

with the Vancouver Island population included, but the correlation was greater and the test more significant when the Vancouver Island sample was removed (R_S = 0.837; $p < 0.001$; Fig. 2, "V" points omitted). Vancouver Island falls markedly off the differentiation-by-distance curve at small distances. This population shows excess differentiation from the Rocky Mountain samples (dashed outline; Fig. 2), and this is attributable to genetic drift in a relatively isolated island population (Tompa 1983).

The high mutation rate and stepwise mutation mechanism at microsatellite loci make genetic distances such as Nei's *D* and F_{ST} increasingly suspect as differentiation increases (Kimmel et al. 1996; Slatkin 1995; Nauta & Weissing 1996). In contiguous subpopulations, where gene flow is high or where separation is very recent, population processes will have a stronger effect than mutation and inferences based on these measures are reliable. The range within which this is true, however, is not well established and may vary among taxa. In the present case, positive correlations in the above tests indicate that isolation-by-distance is measurable between wolf subpopulations if samples are sufficiently numer-

ous and large and if the tested populations cover a sufficient range of distances. The lack of differentiation-distance correlation found by Roy et al. (1994) may be due to absence of migration-drift equilibrium or to homoplasy accumulated due to back-mutation at large genetic divergences. However, lower statistical power due to smaller sample sizes, fewer populations, and a smaller range of geographic distances could also be responsible. Furthermore, inclusion of an island population may have obscured a pattern of migration-drift equilibrium on the rest of the continent.

Genetic Estimates of Dispersal

Inferring reliable estimates of gene flow due to migration of individuals between populations is one of the most difficult problems in conservation biology (Varvio et al. 1986; Avise 1994). Because genetic estimates of migration are suspect where the evidence for migration-drift equilibrium is weak or lacking (Slatkin 1993), the most reliable estimates will be based on populations most likely to be in equilibrium based on independent information. For this purpose Fort St. John, Hinton, and the Northwest Territories are the best choices because (1) wolves were never fully extirpated from these areas (Gunson 1983; Heard 1983; Tompa 1983), so there is not a recent history of recolonization in these areas; (2) these populations are close enough together (600–1200 km) and in adequately continuous wolf habitat to provide potential gene flow by migration based on field data; and (3) divergence between contiguous populations will be least affected by high microsatellite mutation rates.

For the Fort St. John and Hinton samples (about 600 km apart), the migration estimate (N_em) was 2.7 migrants per generation (N_e is the effective population size, and m is the proportion of the population that is migrants each generation; Slatkin 1987). Between the Northwest Territories and either Fort St. John or Hinton (about 1000 and 1200 km respectively) the estimates are correspondingly less: $N_em = 1.6$ and 2.3 migrants per generation for the Northwest Territories/Fort St. John for the Northwest Territories/Hinton, respectively. Given that the error in genetically estimating migration may be 20 to 100% (Slatkin & Barton 1989), all the above results are in reasonable agreement. These estimates are expressed as the absolute number of migrants between populations, independent of population size. Thus, in a population of 100 packs (200 breeding adults) two migrants per generation would mean replacement of only 1% of the breeding adults each generation.

Evidence of Dispersal from Field Studies

Because field and genetic data differ in their ability to estimate historical versus current gene flow, a combination of these approaches is advisable (Slatkin 1987; Avise 1994). Our field data corroborate that the genetically estimated rate of two or more migrants per generation is reasonable. The field evidence of migration rates in Rocky Mountain wolves comes from an intensive study of dispersal in the Glacier National Park (GNP) area recolonizing population, where high migration rates and migration distances ranging from 200 to over 800 km are reported (Ream et al. 1991; Boyd et al. 1995). These are comparable to reports of long-distance wolf dispersal in other areas such as Minnesota where human development of the landscape is substantial (Gese & Mech 1991; Mech et al. 1995). There is no cumulative evidence of sex bias in dispersal frequency or distance in these studies.

These large dispersal distances and rates suggest that movements among widely separated packs and among the three recovery areas are likely and that two migrants per generation between large, permanent wolf populations is possible. Distances between the population centers of the three recovery areas range from 370 km between YNP and central Idaho to 540 km between GNP and YNP (Fig. 1), and these distances are readily traversed by wolves when conditions are favorable. Southward breeding dispersal of wolves from GNP has already covered about half the distance from GNP to each of the two reintroduction sites (shaded area extending south of GNP; Fig. 1), and dispersal movements of Idaho wolves have already ranged near the natural colonization area (Fritts et al. 1997).

Management for Wolf Migration

The mountainous character of the study area fragments the landscape into patches of suitable wolf habitat, usually centered around lower elevation valleys, in a matrix of unsuitable habitat. This precludes the existence of a continuous population of boundary-sharing packs, and it encourages dispersal and consequent gene flow among regions. If truly isolated in mountain valleys, these wolf packs might potentially suffer inbreeding depression. The long-distance movements described here, however, show that such isolation is very unlikely.

Generalizations drawn from studies of permanent populations in more homogeneous habitat (e.g., northern Minnesota, parts of Canada and Alaska) may not apply to expanding populations in heterogeneous, mountainous habitat. Patchy habitat distribution may make Rocky Mountain wolves more typical of wolves in human-affected landscapes, where populations become increasingly fragmented as development intrudes. Human interference (ranches, highways, poachers) rather than absolute distance will most likely limit migration between recovery areas. These obstructions, as well as political status and social attitudes, vary spatially and temporally throughout our international study area, but are

nevertheless key factors in wolf conservation (Mech 1995; Fritts & Carbyn 1995).

Conservation planning includes enhancing genetic exchange among recovery areas by management for migration corridors. The effectiveness of corridors, however, depends on the needs and behaviors of individual species (Noss et al. 1996). Wolves disperse at much greater rates and over longer distances than other large carnivores, and they may be less prone to avoid human development when habitat quality is otherwise high (Mech 1995; Mech et al. 1995; Paquet et al. 1996). Neither do wolves necessarily choose designated recovery lands (U.S. Fish and Wildlife Service 1994) for habitation. Seven of the 15 breeding packs recorded during natural recolonization (Fortine, Marion, Ninemile, Boulder, Thompson River, Browning, and Choteau) were established both outside the recovery area and outside suggested wildlife migration corridors (U.S. Fish and Wildlife Service 1987). Because wolves disperse so effectively, planning for discrete corridors may be less important than management for wolf survival in the broad landscape linkages already in use by wolves (Fritts & Carbyn 1995; Noss et al. 1996). In the Rocky Mountains these connections are diminishing but apparently adequate at present.

Genetic Aspects of Wolf Recovery

Reintroduced populations are generally small, and genetic principles must be considered in their management (Leberg 1990). The goal is to choose founders so as to avoid loss of genetic variation, which in general means using as many unrelated animals of both sexes as possible from a population with a high level of variation. In social animals, however, effects of management disruption on pair bonds and reproductive timing must also be considered. Prescriptions for wolf reintroduction call for use of animals from the closest thriving population to minimize outbreeding and loss of local adaptation and the transfer of extant packs to promote early reproduction (Shields 1983; U.S. Fish and Wildlife Service 1994).

The 1995 and 1996 reintroductions followed these guidelines (Fritts et al. 1997), and the result has been beneficial from a genetics perspective. The two genetically distinct source populations had high heterozygosity levels, and the mixing of these sources was additionally beneficial.

Genetic variation in the reintroduced populations is substantial and the initial population size is apparently adequate to prevent a small founding bottleneck (N = 31 and 35 in YNP and Idaho, respectively). However, a founder effect is still inevitable in the first generations of reproduction. Heterozygosity is expected to be lost at a rate of $1/(2N_e)$ per generation where N_e is the effective population size (Wright 1969), and in wolves N_e is much less than the census population size due to the limitation of breeding to alpha pairs (Chepko-Sade et al. 1987). The severity of the founding bottleneck will depend on the initial rate of reproduction and ongoing survivorship. However, because the founding stock had high levels of genetic variation, the immediate concern is more about short-term demography than about genetics (Lande 1988). These demographic factors are difficult to predict and are confounded by the uncertainties of human-caused mortality.

The naturally recolonized Montana population potentially remains connected by migration with Canada. Thus, dispersal among the YNP and Idaho reintroduction areas and the recolonized Montana population could connect the U.S. and Canadian Rocky Mountain populations. Gene flow throughout the Rocky Mountains would ultimately connect the reintroduced U.S. populations to a large Canadian metapopulation that numbers in the tens of thousands. Artificial translocation is also seen as a viable option if natural migration is inadequate (U.S. Fish and Wildlife Service 1994).

Conclusions

It appears that all Rocky Mountain wolves, whether they are in permanent, recovered, or reintroduced populations, have high heterozygosity ultimately because of the dispersal of genetically sufficient numbers of animals from stable population centers. We conclude that none of the three recovery populations in isolation would necessarily maintain a genetically viable population in the long run, but that the dispersal capabilities of wolves make such isolation unlikely if populations remain near recovery goals. A greater threat to wolf recovery is the possibility of chronically low numbers or minimal dispersal due to human-caused mortality. Broad landscape connections where wolves are not persecuted outside designated recovery areas are needed, and these can be enhanced through effective legal protection and public education.

A combination of field work and genetic analysis yields valuable knowledge of wolves that neither of these approaches alone can provide. The finding of high genetic variation obviates any immediate concerns about inbreeding in Rocky Mountain wolves. However, these same field and laboratory techniques will be needed in the future to assess population numbers and long-term effective population size and to identify dispersers as members of the natural population.

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Literature Cited

Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences **38:**1507–1514.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Bangs, E. E., and S. H. Fritts. 1996. Reintroducing the gray wolf to central Idaho and Yellowstone National Park. Wildlife Society Bulletin **24:**402–413.

Boyd, D. K., P. C. Paquet, S. Donelon, R. R. Ream, D. H. Pletscher, and C. C. White. 1995. Transboundary movements of a recolonizing wolf population in the Rocky Mountains. Pages 135–140 in L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, Alberta.

Chepko-Sade, B. D., W. M. Shields, J. Berger, Z. T. Halpin, W. T. Jones, L. L. Rogers, J. P. Rood, and A. T. Smith. 1987. The effects of dispersal and social structure on effective population size. Pages 287–321 in B. D. Chepko-Sade and Z. T. Halpin, editors. Mammalian dispersal patterns. University of Chicago Press, Chicago.

Cockerham, C. C., and B. S. Weir. 1993. Estimation of gene-flow from F-statistics. Evolution **47:**855–863.

Forbes, S. H., and D. K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the Central Rocky Mountains. Conservation Biology **10:**1082–1090.

Fritts, S. H., and L. N. Carbyn. 1995. Population viability, nature reserves, and the outlook for gray wolf conservation in North America. Restoration Ecology **3:**26–38.

Fritts, S. H., E. E. Bangs, J. A. Fontaine, W. G. Brewster, and J. F. Gore. 1995. Restoring wolves to the northern Rocky Mountains of the United States. Pages 107–125 in L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, Alberta.

Fritts, S. H., E. E. Bangs, J. A. Fontaine, M. R. Johnson, M. K. Phillips, E. D. Koch, and J. R. Gunson. 1997. Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. Restoration Ecology **5:**7–27.

Garcia-Moreno, J., M. D. Matocq, M. S. Roy, E. Geffen, and R. K. Wayne. 1996. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. Conservation Biology **10:**376–389.

Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Canadian Journal of Zoology **69:**2946–2955.

Gottelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman, J. Garcia-Moreno, E. A. Ostrander, and R. K. Wayne. 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. Molecular Ecology **3:**310–312.

Gunson, J. R. 1983. Status and management of wolves in Alberta. Pages 25–29 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Gunson, J. R. 1992. Historical and present management of wolves in Alberta. Wildlife Society Bulletin **20:**330–339.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Heard, D. C. 1983. Historical and present status of wolves in the Northwest Territories. Pages 44–47 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Kennedy, P. K., M. L. Kennedy, P. L. Clarkson, and I. S. Liepins. 1991. Genetic variability in natural populations of the gray wolf, *Canis lupus*. Canadian Journal of Zoology **69:**1183–1188.

Kimmel, M., R. Chakraborty, D. N. Stivers, and R. Deka. 1996. Dynamics of repeat polymorphisms under a forward-backward mutation model: within- and between-population variability at microsatellite loci. Genetics **143:**549–555.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241:**1455–1460.

Leberg, P. L. 1990. Genetic considerations in the design of introduction programs. Transactions of the North American Wildlife and Natural Resources Conference **55:**609–619.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution **46:**477–494.

Mech, L. D. 1995. The challenge and opportunity of recovering wolf populations. Conservation Biology **9:**270–278.

Mech, L. D., S. H. Fritts, and D. Wagner. 1995. Minnesota wolf dispersal to Wisconsin and Michigan. American Midland Naturalist **133:**368–370.

Nauta, M. J., and F. J. Weissing. 1996. Constraints on Allele size at microsatellite loci: implications for genetic differentiation. Genetics **143:**1021–1032.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Annals of Human Genetics **41:**225–233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:**583–590.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Noss, R. F., H. B. Quigley, M. G. Hornocker, T. Merrill, and P. C. Paquet. 1996. Conservation biology and carnivore conservation in the Rocky Mountains. Conservation Biology **10:**949–963.

Nowak, R. M. 1983. A perspective on the taxonomy of wolves in North America. Pages 10–19 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Ostrander, E. A., G. F. Sprague, and J. Rine. 1993. Identification and characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. Genomics **16:**207–213.

Paquet, P. C. 1993. Summary reference document - ecological studies of recolonizing wolves in the Central Canadian Rocky Mountains. Banff National Park Warden Service, Banff, Alberta.

Paquet, P. C., J. Wierchowski, and C. Callaghan. 1996. Effects of human activities on gray wolves in the Bow River Valley Banff National Park, Alberta—a summary. In J. Green, C. S. Pacas, L. Bayley, and L. Cornwell, editors. Ecological outlooks project: a cumulative effects assessment and futures outlook of the Banff Bow Valley. Department of Canadian Heritage, Ottawa, Ontario.

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86:**248–249.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf

population. Pages 349–366 in R. B. Keiter and M. S. Boyce, editors. The greater Yellowstone ecosystem: redefining America's wilderness heritage. Yale University Press, New Haven, Connecticut.

Roy, M. S., E. Geffen, D. Smith, E. A. Ostrander, and R. K. Wayne. 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution **11:**553–570.

Shields, W. M. 1983. Genetic considerations in the management of the wolf and other large vertebrates. Pages 90–92 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science **236:**787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution **47:**264–279.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:**457–462.

Slatkin, M., and N. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution **43:**1349–1368.

Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign.

Tompa, F. S. 1983. Status and management of wolves in British Columbia. Pages 20–24 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

U.S. Fish and Wildlife Service. 1987. Northern Rocky Mountain wolf recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado.

U.S. Fish and Wildlife Service. 1994. Memorandum regarding a viable wolf population in the Northern Rocky Mountains. Pages 6–68 to 6–78 in Final environmental impact statement: the reintroduction of gray wolves to Yellowstone National Park and central Idaho. U.S. Fish and Wildlife Service, Denver, Colorado.

Varvio, S., R. Chakraborty, and M. Nei. 1986. Genetic variation in subdivided populations and conservation genetics. Heredity **57:**189–198.

Wright, S. 1931. Evolution in Mendelian populations. Genetics **16:** 97–259.

Wright, S. 1969. Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago.

Appendix

Allele frequencies at 10 microsatellite loci in Rocky Mountain wolves.

		Population (sample size)					
Locus and allele[a]	BP[b]	*Fort St. John (41)*	*Hinton (33)*	*Banff (32)*	*Montana (66)*	*Yellowstone (31)*	*Idaho (35)*
2							
D	213	0.500	0.409	0.766	0.705	0.387	0.486
E	215	0.061	0.015	0.000	0.000	0.048	0.043
F	217	0.402	0.030	0.000	0.129	0.274	0.229
H	221	0.000	0.167	0.031	0.000	0.048	0.114
I	223	0.037	0.379	0.203	0.167	0.242	0.129
109							
A	143	0.012	0.348	0.094	0.030	0.226	0.129
B	145	0.427	0.136	0.203	0.212	0.274	0.314
C	147	0.024	0.227	0.281	0.159	0.161	0.086
D	149	0.305	0.106	0.031	0.250	0.161	0.271
E	151	0.122	0.061	0.375	0.303	0.048	0.086
F	153	0.012	0.000	0.000	0.000	0.016	0.000
G	155	0.098	0.121	0.016	0.045	0.113	0.114
123							
E	145	0.780	0.727	0.563	0.712	0.758	0.729
F	147	0.000	0.000	0.000	0.008	0.000	0.000
G	149	0.037	0.061	0.172	0.182	0.016	0.086
H	151	0.183	0.015	0.000	0.000	0.097	0.114
I	153	0.000	0.000	0.063	0.000	0.000	0.000
J	155	0.000	0.197	0.203	0.098	0.129	0.071
172							
H	155	0.488	0.485	0.141	0.288	0.516	0.457
I	157	0.512	0.515	0.859	0.712	0.484	0.543
200							
E	123	0.268	0.485	0.656	0.333	0.387	0.343
I	131	0.268	0.091	0.031	0.235	0.161	0.214
J	133	0.195	0.303	0.156	0.318	0.194	0.286
K	135	0.012	0.030	0.000	0.000	0.048	0.000
L	137	0.256	0.091	0.156	0.114	0.210	0.157
204							
A	202	0.049	0.197	0.281	0.318	0.097	0.143
B	204	0.085	0.242	0.344	0.129	0.177	0.157
D	208	0.317	0.333	0.344	0.318	0.339	0.300

Appendix. Continued

Locus and allele[a]	BP[b]	*Population (sample size)*					
		Fort St. John (41)	*Hinton (33)*	*Banff (32)*	*Montana (66)*	*Yellowstone (31)*	*Idaho (35)*
E	210	0.549	0.227	0.031	0.235	0.387	0.400
225							
B	160	0.354	0.424	0.078	0.235	0.452	0.314
C	162	0.378	0.379	0.500	0.288	0.355	0.443
D	164	0.244	0.000	0.141	0.045	0.113	0.143
E	166	0.024	0.197	0.281	0.432	0.081	0.100
250							
E	134	0.000	0.000	0.063	0.000	0.000	0.000
F	136	0.244	0.197	0.250	0.182	0.258	0.171
G	138	0.183	0.273	0.047	0.053	0.290	0.200
H	140	0.232	0.348	0.313	0.076	0.161	0.386
I	142	0.000	0.015	0.063	0.205	0.000	0.000
J	144	0.232	0.136	0.266	0.485	0.226	0.143
L	148	0.110	0.030	0.000	0.000	0.065	0.100
344							
A	156	0.913	0.818	0.734	0.697	0.883	0.871
D	162	0.050	0.030	0.063	0.061	0.050	0.043
E	164	0.013	0.152	0.172	0.242	0.050	0.071
F	166	0.000	0.000	0.031	0.000	0.000	0.000
G	168	0.025	0.000	0.000	0.000	0.017	0.014
377							
B	146	0.073	0.076	0.000	0.023	0.000	0.129
C	148	0.098	0.136	0.141	0.500	0.145	0.114
G	156	0.049	0.000	0.094	0.000	0.032	0.029
H	158	0.037	0.045	0.016	0.045	0.048	0.014
I	160	0.000	0.045	0.000	0.000	0.032	0.014
J	162	0.134	0.439	0.203	0.144	0.242	0.314
K	164	0.122	0.061	0.063	0.045	0.129	0.086
L	166	0.488	0.197	0.438	0.242	0.371	0.300
O	172	0.000	0.000	0.047	0.000	0.000	0.000

[a] *Letter codes designate two-basepair allele size increments that match the codes in Roy et al. (1994).*
[b] BP *is the size of the PCR product for each allele in DNA basepairs.*