

Genetic Evaluation of the Three Captive Mexican Wolf Lineages

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The Mexican gray wolf appears to be extinct in the wild and exists now only in captivity. There are three captive putative Mexican wolf lineages: Certified, Ghost Ranch, and Aragón lineages. From pedigree analysis, the Certified lineage has the lowest level of inbreeding and has retained the most founder alleles. In contrast, the Ghost Ranch has a high level of inbreeding and fewest founder alleles. From molecular genetics data, particularly from microsatellite loci, it appears that all of the lineages consist of Mexican wolves, and none of them appear to have ancestry from dogs or coyotes. Further, the molecular data are consistent with the Certified lineage having three founders instead of the four previously assumed. From these findings, we recommend that the three lineages be combined to increase the number of founders and to postpone any inbreeding depression. The combination of pedigree analysis and molecular genetic data allowed us to resolve several long-standing concerns in Mexican wolves. *Zoo Biol* 16:47–69, 1997. © 1997 Wiley-Liss, Inc.

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INTRODUCTION

A number of species or subspecies are now extinct in the wild and are only preserved in captive populations. Often the genetic foundation of captive endangered populations is limited because only a few individuals were still alive or available when the captive population was initiated. For example, the captive population of Speke's gazelle has only four founders, the black-footed ferret only six founders, and Przewalski's horse only 13 founders. In cases in which the founder number for a captive lineage is small, it is important whenever possible to increase the number of founders, both to increase the extent of genetic variation in the population and to

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avoid the detrimental effects of inbreeding. However, before incorporating new founders, it is crucial to be certain that the new founders are of the same subspecies or species and to determine that there is no ancestry from other subspecies or species in the potential new founders. We discuss such an example in the Mexican gray wolf in which the Certified captive population descends from three individuals (see below), but there are two other captive putative Mexican wolf lineages, each thought to be descended from two other independent founders.

The gray wolf, *Canis lupus*, had a distribution throughout most of North America before eradication programs, starting in late nineteenth century, resulted in extirpation from most of its original range. The remaining wolves were listed as endangered by the US Fish and Wildlife Service, and the Mexican gray wolf subspecies, *Canis lupus baileyi*, was listed as endangered in 1976. Mexican wolves are thought to have been extirpated from the United States and greatly reduced in Mexico by 1970 [Brown, 1983] (there is no confirmed evidence of wild Mexican wolves in Mexico for the last decade, and they are now thought to be extinct in the wild throughout their ancestral range). As a result, wolves from Mexico were captured in the late 1970s and taken to the United States to start a captive breeding program. These wolves are the foundation of what has been known as the Certified lineage of Mexican wolves. In addition, two other lineages, the Ghost Ranch (also known as Arizona-Sonora Desert Museum/Ghost Ranch or ASDM/GR) and the Aragón lineage, are both thought to be founded from Mexican wolves. However, there has been some question about the founders and their ancestry in both these lineages (see below), so they were not included in the Certified Mexican wolf lineage.

We first summarize the history and pedigree information for the three captive Mexican wolf lineages. Next, we briefly discuss the morphological (mainly skull) characteristics of the three lineages. Finally, we summarize the molecular genetics data on the three lineages and discuss in detail the microsatellite data. From this information, particularly the microsatellite data, we conclude that all three lineages are indeed Mexican wolves. Using the combination of pedigree and molecular genetic information, we recommend a protocol for incorporating the Ghost Ranch and Aragón lineages into the Certified lineage. The incorporation of the two additional lineages into the captive Mexican wolf population should both increase the number of founders, and thereby the amount of genetic variation, and reduce the likelihood of inbreeding depression in these animals.

CAPTIVE LINEAGES AND PEDIGREES

Certified Lineage

The Certified lineage of Mexican wolves consists of individuals whose lineage can be traced to wild-caught Mexican stock [US Fish and Wildlife Service, 1981]. Six wolves (five males and one pregnant female) were captured in the Mexican states of Durango (four wolves) and Chihuahua (two wolves) from 1972–1980 and placed in captivity in the United States [Siminski, 1993]. No wolves have been removed from the wild since 1980. The mate of the pregnant female, presumed to be unrelated to the other wild-caught males, was not captured. Two of the captured males (studbook numbers 2 and 11), the captured pregnant female (5), and her wild mate (referred to as P5) founded the Certified lineage [Siminski, 1993]. At the time of capture, male

2 appeared to be approximately 6 months old and was not thought to be of reproductive age. It now appears that male 2 was an offspring of the wild-caught female and her wild mate in the year previous to capture [Wayne, 1995] (see section below on number of founders). We will assume in the pedigree below that there are only three independent founders (5, 11, and P5) for the Certified lineage based on the genetic and other evidence outlined below. This is different than the scenario used previously in which it was assumed that there were four independent founders. The Certified lineage has been professionally managed since its inception for maximum retention of genetic variation and founder representation [American Zoo and Aquarium Association, 1994].

Figure 1 gives the pedigree as of July, 1994, of the Certified lineage where the founders are represented on the top line. The small closed circles are mating nodes, and, for example, the node below 5 and P5 leads to their progeny 2, 10, etc. (see the figure legend for information about the symbols used). As of July, 1994, the Certified population numbered 91 living animals (indicated by closed symbols in Fig. 1), 77 in the United States and 14 in Mexico. From analysis of this pedigree, the average inbreeding coefficient of the living wolves is 0.184 (this and other values for the three lineages are summarized in Table 8). In addition, if we assume that all the alleles in the three founders are not identical by descent at a given locus, then, given the structure of the pedigree in Figure 1, it is theoretically expected from analyzing the pedigree that on average 5.41 alleles (or 90.2% of the original six alleles) per locus would be remaining in the living population. In addition, there is no indication of inbreeding depression for either juvenile survival or body size in the Certified lineage [Miller and Hedrick, unpublished].

Ghost Ranch Lineage

All of the Ghost Ranch lineage wolves are currently in the United States and descend from two founders. The founding male was trapped alive in 1959 in the Tumacacori Mountains of southern Arizona, approximately 10 miles north of the Arizona-Sonora border. It was described by some observers as looking like a wolf-dog hybrid. Carley [1979] described the animal's appearance as that of a "wolf-like" canid. The founding female was claimed to be a wild-caught pup taken near Yecora, Sonora, in 1961 and is generally assumed to be a pure Mexican wolf. Remains of the two founders are not available for analysis. Management of this lineage has been haphazard, poorly documented, and characterized by extensive full-sib mating [Carley, 1979]. Although Carley [1979] described this lineage as highly inbred, Ames [1980] concludes that "inbreeding does not appear to have significantly affected" this lineage. A quantitative evaluation of this observation has not been carried out and may not be possible because detailed data of juvenile mortality and other fitness components are not available.

The pedigree for the Ghost Ranch lineage is given in Figure 2 using the same symbols as in Figure 1. Eighteen animals from this lineage are alive, 13 of which are indicated in Figure 2 [Siminski, personal communication]. The inbreeding coefficient in the living individuals, 0.608, is quite high. If we assume that all the alleles in the two founders were not identical by descent, it is expected from pedigree analysis only 2.02 alleles (or 50.5% of the original number) per locus are surviving in the living animals.

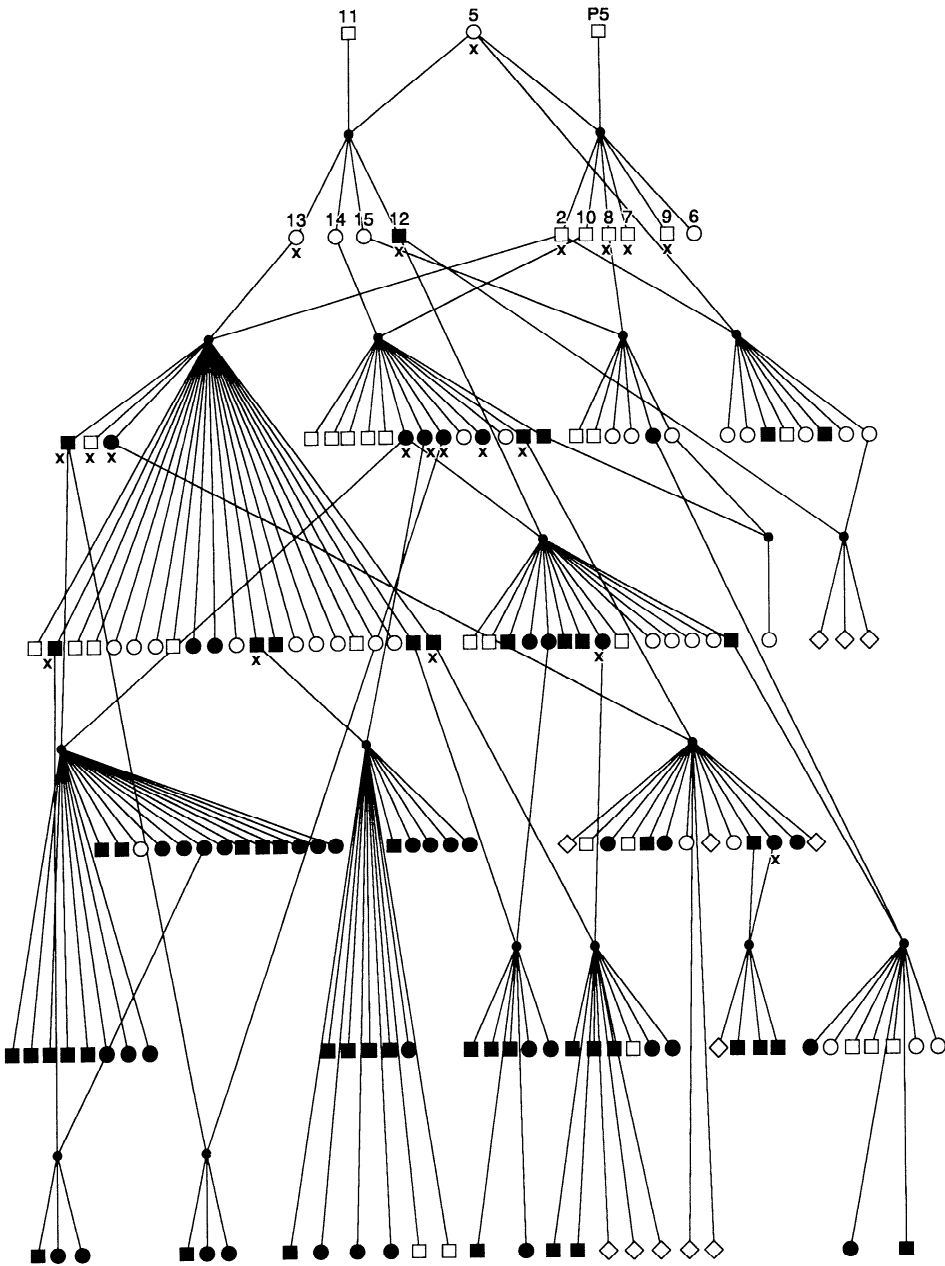


Fig. 1. The pedigree of the Certified lineage as of July, 1994, with squares, circles, and diamonds indicating males, females, and wolves of unknown sex, respectively. Solid and open symbols indicate living and dead wolves, respectively. The wolves sampled by García-Moreno et al. [1996] and Wayne [1995] for microsatellite variation are indicated by an x.

Aragón Lineage

The Aragón lineage has been maintained at the San Juan de Aragón Zoo in Mexico City since 1965, and all are presently in Mexico. Founders of this lineage

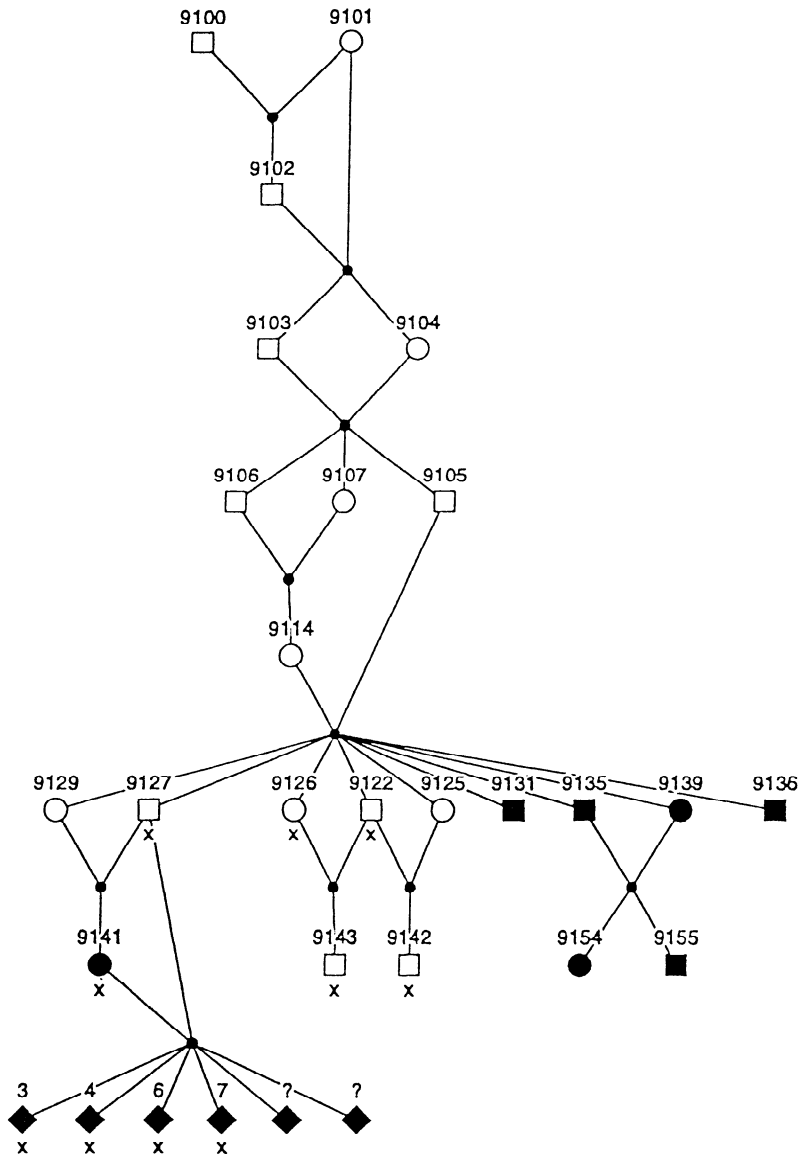


Fig. 2. The pedigree of the Ghost Ranch lineage, with squares, circles, and diamonds indicating males, females, and wolves of unknown sex, respectively. Solid and open symbols indicate living and dead wolves, respectively. The wolves sampled by García-Moreno et al. [1996] and Wayne [1995] for microsatellite variation are indicated by an x.

were obtained from the Chapultepec Zoo in Mexican City on two separate occasions, but the origin of the Chapultepec stock is unknown. The number of founders is thought to be two or perhaps three [Gerardo Lopez, personal communication]. In 1986, two Aragón lineage females were bred by a presumed wolf-dog hybrid (Zico); however, Zico and his offspring were separated from the Aragón group in 1987. No subsequent interbreeding of the hybrids and Aragón group occurred.

The pedigree for the Aragón lineage is given in Figure 3, with the eight animals that currently comprise this lineage indicated by closed symbols. The early history of this lineage is not known, and, for example, the date and location of the capture of the founders are unknown. Further, it is likely that the founders given in Figure 3 (–01 and –02) are themselves inbred, but there is no information to substantiate this one way or the other. Given the pedigree in Figure 3, the average coefficient of inbreeding in the living individuals is 0.263, and the expected number of alleles per locus surviving, assuming that the initial four alleles were not identical by descent, is 3.44, or 86% of the initial number. It is assumed here that female 8 is the mother of the litter with individuals 1–6, but it is possible that female 7, her sister, is the mother of this litter. In this case, the average coefficient of inbreeding is slightly lower at 0.200, and the number of alleles surviving is the same at 3.44. It is also assumed that –02 is the mother of both –23 and 8. It is also possible that another female founder, –03, was the mother of 7 and 8 [Gerardo Lopez, personal communication]. In this case, the inbreeding coefficient would be lower and the number of surviving alleles would be higher than given for the pedigrees above.

MORPHOLOGICAL ANALYSES

The Mexican wolf is one of the smaller subspecies of the gray wolf in North America, with adult weight between 23 and 41 kg, total length between 180 and 220 cm, and height at the shoulder between 75 and 105 cm. However, there is a great range in size in both Mexican wolves and other subspecies of gray wolves, so there is substantial overlap in sizes between subspecies.

Bogan and Mchlhop [1983] compared 25 skull measurements for gray wolves from southwestern North America, dogs, and coyotes. Among the skulls examined were eight specimens from the Ghost Ranch lineage. They classified seven of these as belonging to the southern wolf groups (central and southern Arizona, southern New Mexico, western Texas, and Mexico) and one as belonging to the northern wolf group (northern New Mexico). Bogan and Mchlhop [1983] concluded that all wolves in their southern group should be considered *Canis lupus baileyi*. In other words, they suggested that the now extinct subspecies, *C. l. mogollonensis* and *C. l. monstabilis*, which had ranges adjacent and mainly to the north of *C. l. baileyi*, be included within *C. l. baileyi*. However, morphological analyses by both Hoffmeister [1986] and Nowak [1995] indicated that *C. l. mogollonensis* and *C. l. monstabilis* may belong to a subspecies other than *C. l. baileyi* and that the natural range of the latter is about the same as originally designated. Bogan and Mchlhop [1983] found that individuals from the Ghost Ranch lineage had relatively shorter rostra than wild-raised wolves, but they were unable to determine if this condition was the result of genetic causes or captive rearing.

Weber [1989] compared the skulls of Aragón wolves to those of Mexican wolves in the collection of the Institute of Biology of the National University of Mexico (UNAM) as well as to those of dogs and coyotes. He found that the Aragón skulls grouped with the Mexican wolves and were clearly separate from dogs and coyotes. Lopez and Vazquez [1991] point out that Weber's [1989] sample size was too small to be definitive but that his results are, nevertheless, suggestive of the purity of the Aragón wolves. Further examination of cranial morphology by Lopez and

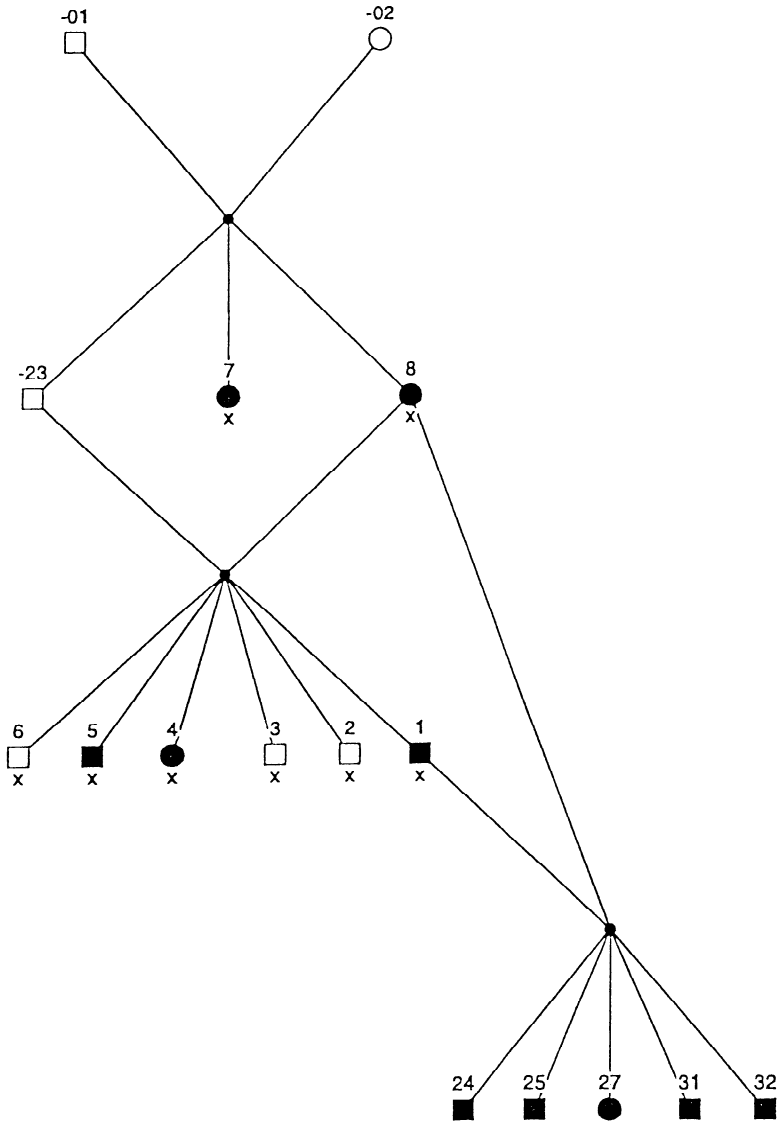


Fig. 3. The pedigree of the Aragón lineage, with squares, circles, and diamonds indicating males, females, and wolves of unknown sex, respectively. Solid and open symbols indicate living and dead wolves, respectively. The wolves sampled by García-Moreno et al. [1996] and Wayne [1995] for microsatellite variation are indicated by an x.

Vazquez [1991] also provided no evidence to indicate hybrid ancestry in the Aragón lineage.

Nowak [personal communication] has examined skulls of the six wild-caught individuals from the Certified lineage (three of these did not contribute to the present lineage). He has concluded that there does not appear to be any evidence of hybridization with other species in these animals. However, two of the males and the single female were smaller and more lightly built than other *C. l. baileyi* he has examined

TABLE 1. Allele frequencies for the three polymorphic allozyme loci found in two of the Mexican wolf lineages, gray wolves, coyotes and dogs (sample size in parentheses) [Shields et al., 1987]

Taxa	<i>Pgi-2</i>		<i>Nsp</i>		<i>Got</i>		
	-100	100	100	110	100	110	120
Certified (30)	0.00	1.00	0.25	0.75	1.00	0.00	0.00
Ghost Ranch (8)	0.00	1.00	0.31	0.69	0.00	1.00	0.00
Gray wolf (19)	0.00	1.00	0.50	0.50	0.00	0.96	0.04
Coyote (25)	0.87	0.13	1.00	0.00	0.00	1.00	0.00
Dog (10)	0.00	1.00	0.00	1.00	0.00	1.00	0.00

while still within the lower limits of the size range for *C. l. baileyi*. Nowak [personal communication] also examined the skulls of some of the other captive animals and has noted certain questionable characters in a few specimens, especially small teeth and bullae, which could hint at influence from the domestic dog. It is quite possible that the smaller size in the two wild-caught males, as well as the unusual characters in the other specimens, may be partly the result of rearing and maintenance in captivity; however, the influence of captive rearing and maintenance on morphological development is generally poorly documented in wolves.

MOLECULAR GENETIC ANALYSES

Allozymes

Shields et al. [1987] examined the allozyme variation at 22 loci in wolves from the Certified and Ghost Ranch lineages, northern gray wolves, dogs, red wolves, and coyotes. For 19 of these loci, all taxa were fixed for the same allele, while three of these loci, *Pgi-2*, *Nsp*, and *Got*, were variable either within or between taxa (Table 1) (red wolf data not given here). *Pgi-2* was monomorphic for the same allele (100) in the Certified, Ghost Ranch, gray wolf, and dog samples and, therefore, was only diagnostic to show that there does not appear to be ancestry in the two Mexican wolf samples from coyotes, which had another allele, -100, in high frequency. *Nsp* was polymorphic for the same two alleles in the Certified, Ghost Ranch, and gray wolf samples and monomorphic in dogs and coyotes, suggesting that the three wolf samples were more closely related to each other than to coyotes and dogs. For *Got*, allele 110 was either fixed or in high frequency in all the taxa except the Certified sample, which was fixed for another allele (100). Shields et al. [1987] concluded that there is a closer relationship between the Certified and Ghost Ranch lineages than either shows to any of the other canids examined. However, the basis for this conclusion is not obvious from these data because the Ghost Ranch lineage appears quite close in allele frequencies to the northern gray wolf sample, obviously closer than to the Certified lineage because of the large difference in the frequency of the *Got* 110 allele.

Although allozymes have been used to determine relationships among taxa in many organisms, they are slowly evolving and often do not have the resolution or the variation to distinguish between closely related taxa. In this case, the small number of founders in these lineages may have resulted in a chance change in allele frequency that led to the difference between the Certified and Ghost Ranch samples at the *Got*

TABLE 2. Difference between the Certified Mexican wolf lineage and other taxa for mtDNA*

	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Shields et al. [1987]	0	—	1–4	15–17	1–4
Wayne et al. [1992]	0.00	—	0.10–0.51	3.06	—
Fain et al. [1995]	0.0	1.2	0.2	8.3	1.8

*The results for Shields et al. [1987] are given as the number of restriction site differences, the results for Wayne et al. [1992] as the estimated percent divergence based on the proportion of shared restriction sites, and the results for Fain et al. [1995] as the percent nucleotide sequence divergence. Comparisons with no data are indicated by a dash.

locus. With just a few variable loci, it is only possible to suggest that the Certified and Ghost Ranch lineages appear to group with gray wolves (with the Ghost Ranch and gray wolves the closest of this group) and that coyotes and dog are more distantly related.

Mitochondrial DNA

Molecular analysis of mitochondrial DNA (mtDNA) can often resolve differences not observable with allozymes because of the faster rate of evolutionary change in mtDNA. However, mtDNA is maternally inherited and therefore does not give an indication of the paternal ancestry of founders. Shields et al. [1987] and Wayne et al. [1992] carried out analysis of mtDNA using restriction enzymes to define haplotypes in Mexican wolves. Shields et al. [1987] and Wayne et al. [1992] identified 55 and 95 restriction sites, thereby surveying approximately 300 and 500 base pairs, respectively, of the 16,800 bases in canid mtDNA. The Certified and Ghost Ranch lineages were both founded by a single female, so only one mtDNA type should be expected in each line. The number of female founders in the Aragón line is not known, but the present animals are thought to be all descended from one female (8) or two sisters (7 and 8) with the same mother, so there should also be only one mtDNA type in this lineage.

Shields et al. [1987] examined mtDNA variation in the Certified and the Ghost Ranch lineages and found that they had an identical haplotype which was different from that in other taxa. This haplotype differed by one restriction site from the next closest haplotype which belonged to both a gray wolf and a dog. Other gray wolf samples and another dog sample differed by more sites, and a sample of coyotes differed by a large number of sites, from the Mexican wolves (see Table 2). Wayne et al. [1992] examined mtDNA from the Certified and Ghost Ranch lineages (no wolves from the Aragón lineage were examined in this study, unlike the statement by Wayne et al. [1992]) and also found that they were identical and, as a group, different from other gray wolf and dog samples. Again, another sample from a gray wolf differed by only one site and coyotes differed by a number of restriction sites from the Mexican wolf haplotype (see Table 2). Both studies demonstrated that gray wolves have extensive mtDNA variation.

Fain et al. [1995] sequenced 576 base pairs of mtDNA, representing part of the highly variable control region, two tRNA genes, and part of the cytochrome b gene, from samples for all three lineages. They found that the Certified and Ghost Ranch animals shared the same haplotype which differed from the Aragón sequence by three substitutions and an 11 base inversion. Again, there was extensive variation among

the gray wolf samples (in this case, one Mexican wolf from each lineage, one northern gray wolf, one Iranian wolf, and one Chinese wolf). The coyote sequence was the most different from the wolves, while the dog sequence was also somewhat different from the wolves (see Table 2).

All three studies indicate that the mtDNA haplotype is the same (or very similar) in the Certified and Ghost Ranch lineages. Further, again from all three studies, it appears quite unlikely that the mtDNA in the Certified and Ghost Ranch lineages has ancestry from coyotes. Other conclusions about the relationship of the Aragón lineage to the other taxa or the relationship of the Mexican wolf taxa to gray wolves or dogs do not appear definitive.

In evaluating these results, it should be realized that the three studies probably sampled different parts of the mitochondrial genome, but the resolution of the sequence data from Fain et al. [1995] should be somewhat higher because it included the highly variable control region. However, the Mexican wolf samples of Fain et al. [1995] included three sequences, one from each lineage, while only one sequence is presented from northern gray wolves, so there is no information on the variability within northern gray wolves. Because sequence information should identify more variation than restriction analysis, one would expect the northern gray wolf, which was variable in the studies of both Shields et al. [1987] and Wayne et al. [1992], to be at least as variable as Mexican wolves using sequence data. In other words, it is likely that northern gray wolves contain a number of sequences (maybe even one similar to the Certified–Ghost Ranch sequence), and it is difficult to determine how significant the difference of 1.2% nucleotide divergence between the Certified–Ghost Ranch sequence and the Aragón sequence is until this information is known. In addition, it is not surprising that two mtDNA haplotypes are present in the three female Mexican wolf founders because the founders were caught from different parts of a wide ancestral distribution.

DNA Fingerprints

Fain et al. [1995] also used multilocus DNA fingerprints to examine variability within and among the three lineages (the sample sizes were as follows: Certified, $N = 33$; Ghost Ranch, $N = 10$; Aragón, $N = 8$). The mean distance, calculated here as $1 - S_{ij}$ (where the S_{ij} values are the similarity values given by Fain et al. [1995]) are for the three comparisons: Certified–Ghost Ranch, 0.54; Certified–Aragón, 0.40; and Ghost Ranch–Aragón, 0.28. In other words, it appears that the Ghost Ranch and Aragón lineages are the most closely related and the Certified and Ghost Ranch are the most distantly related. This is the reverse of the relationship found for the mtDNA by Fain et al. [1995]. Fain et al. [1995] did not compare the DNA fingerprints of the Mexican wolf lineages to other taxa.

Microsatellite Variation

Microsatellite loci have alleles that consist of a variable number of tandem repeats of short sequences that evolve through a gain or loss of repeat units. They have a number of advantages when compared to other DNA markers and are presently the loci of choice to use in identifying differences between closely related taxa. The main advantages are that microsatellite loci are highly variable, fast evolving, codominant, and nuclear and can be analyzed with standard population genetic statistics because alleles and genotypes are identifiable [e.g., Bruford and Wayne, 1993].

García-Moreno et al. [1996] examined variation at ten microsatellite loci, and Wayne [1995] sampled ten additional loci [for primer sequences see Ostrander et al., 1993] in samples of Certified ($N = 20$), Ghost Ranch ($N = 10$), and Aragón ($N = 8$) wolves. Included here for comparison are samples of gray wolves ($N = 55$), coyotes ($N = 39$), and dogs ($N = 27$; one individual from 27 different breeds, including a Siberian husky, an Alaskan husky, and a German shepherd). Table 3 gives the frequencies of all the alleles found in the three Mexican wolf lineages for these 20 microsatellite loci. First, notice that the Certified lineage is polymorphic at 18 of the 20 loci, while Ghost Ranch and Aragón are polymorphic at only nine of the loci (see Table 8 for a summary of these values). The Certified lineage has the highest average number of alleles per locus, 2.50, while both the Ghost Ranch and Aragón lineages have only 1.55 and 1.50 alleles per locus. In addition, the Certified lineage has the highest observed average heterozygosity, 0.457, while the Ghost Ranch and Aragón lineages have observed average heterozygosities of 0.128 and 0.255, respectively.

Given in boldface in Table 3 are the alleles that are most diagnostic of the difference between the Mexican wolf lineages and the other three taxa. For example, locus 172 has an allele G in very high frequency in all the Mexican wolf lineages, and this allele is either at low frequency or absent in gray wolves, coyotes, and dogs. Overall, alleles 109C, 123H, 172G, 213L, 225C, 250G, 253D, 383G, 431I, and 442B are in substantially higher frequency in all three Mexican wolf lineages than in any of the other taxa. There are also a number of alleles that are in substantially higher frequency in two of the three Mexican wolf lineages than in gray wolves, coyotes, and dogs (also in boldface in Table 3). Overall, there are apparent similarities between the three Mexican wolf lineages and substantial differences between the Mexican wolf lineages and the other taxa.

These data can be quantified in several different ways. First, Table 4 gives the genetic distance between the three Mexican wolf lineages, northern gray wolves, coyotes, and dogs, using the genetic distance measure of Nei [1978]. Examining these values, the genetic distances are smallest among the three Mexican wolf lineages, ranging from 0.154–0.393. Notice that the rankings of these three values are the same as that found for the multilocus DNA fingerprints (i.e., the Ghost Ranch–Aragón distance is the smallest and the Certified–Ghost Ranch is the largest). The larger values for the Certified–Ghost Ranch and the Certified–Aragón comparisons may be in part due to the fixation of alleles at 11 of the 20 loci in both the Ghost Ranch and Aragón lineages. The genetic distance of the Mexican wolf lineages with the other three taxa is greater than any of the distances between Mexican wolf lineages.

Figure 4 uses the allele frequencies for the Mexican wolf lineages and dogs, given in Table 3, and allele frequencies for a number of populations of northern gray wolves and coyotes to show how these taxa separate in a multidimensional scaling analysis [Borg, 1981]. Notice in this two-dimensional representation of allele frequency variation that the Mexican wolf lineages cluster together to the right, away from the other taxa, indicating that they share the closest common ancestry and are distinct from the other taxa. The coyote populations from throughout the United States cluster to the left, the northern gray wolf populations from the United States and Canada to the lower center, and the dog to upper center. The large differences here and for the genetic distances in Table 4 between the Mexican wolf lineages and

TABLE 3. Frequencies of all the alleles found in the Mexican wolf lineages [after García-Moreno et al., 1996; Wayne, 1995]*

Gene	Allele	Certified	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
109	B	0.600	0.600	0.000	0.290	0.167	0.333
	C	0.250	0.400	0.313	0.110	0.033	0.000
	F	0.150	0.000	0.688	0.100	0.133	0.250
123	E	0.500	0.100	0.563	0.510	0.086	0.130
	H	0.500	0.900	0.438	0.029	0.243	0.239
147	A	1.000	1.000	1.000	0.813	0.260	0.571
172	G	0.825	1.000	1.000	0.076	0.000	0.000
	H	0.125	0.000	0.000	0.467	0.320	0.000
	I	0.050	0.000	0.000	0.413	0.160	0.944
173	D	0.972	0.100	0.375	0.276	0.000	0.095
	E	0.028	0.600	0.000	0.245	0.027	0.000
	F	0.000	0.150	0.625	0.061	0.095	0.238
	G	0.000	0.150	0.000	0.255	0.000	0.119
200	E	0.650	0.000	0.000	0.114	0.103	0.185
	F	0.350	0.000	0.375	0.148	0.059	0.130
	K	0.000	1.000	0.625	0.011	0.000	0.000
204	A	0.211	0.000	0.000	0.300	0.909	0.396
	B	0.158	0.000	0.000	0.067	0.091	0.021
	D	0.500	1.000	0.813	0.467	0.000	0.021
	E	0.132	0.000	0.188	0.144	0.000	0.000
213	E	0.075	0.000	0.000	0.000	0.017	0.000
	L	0.925	1.000	1.000	0.408	0.167	0.083
225	B	0.025	0.200	0.250	0.244	0.138	0.481
	C	0.975	0.800	0.750	0.378	0.517	0.019
250	E	0.450	0.000	0.000	0.156	0.024	0.000
	G	0.550	1.000	1.000	0.083	0.071	0.204
253	D	1.000	1.000	1.000	0.448	0.088	0.120
344	A	0.350	0.000	0.000	0.688	0.660	0.000
	B	0.650	1.000	1.000	0.135	0.320	0.942
366	H	0.250	0.000	0.000	0.011	0.107	0.000
	I	0.679	0.000	0.000	0.106	0.125	0.094
	L	0.071	1.000	1.000	0.021	0.036	0.094
377	B	0.000	1.000	0.813	0.098	0.019	0.038
	L	0.500	0.000	0.188	0.333	0.111	0.000
	R	0.375	0.000	0.000	0.000	0.000	0.000
	S	0.125	0.000	0.000	0.000	0.000	0.000
383	F	0.000	0.150	0.400	0.302	0.232	0.217
	G	0.500	0.850	0.600	0.271	0.232	0.370
	H	0.071	0.000	0.000	0.104	0.179	0.391
	I	0.429	0.000	0.000	0.063	0.071	0.022
410	C	0.053	1.000	1.000	0.260	0.167	0.105
	D	0.632	0.000	0.000	0.229	0.167	0.132
	F	0.316	0.000	0.000	0.031	0.167	0.132
431	B	0.100	0.000	0.000	0.000	0.000	0.563
	I	0.400	0.500	1.000	0.117	0.063	0.000
	M	0.500	0.500	0.000	0.096	0.000	0.000
442	B	0.789	0.700	1.000	0.435	0.290	0.021
	C	0.211	0.300	0.000	0.185	0.274	0.667
453	A	0.237	0.000	0.500	0.214	0.000	0.071
	I	0.447	0.050	0.400	0.000	0.097	0.238
	J	0.316	0.950	0.100	0.020	0.125	0.000
606	A	0.658	0.350	1.000	0.073	0.019	0.354
	B	0.289	0.000	0.000	0.135	0.019	0.188
	C	0.053	0.000	0.000	0.022	0.241	0.021
	D	0.000	0.650	0.000	0.427	0.056	0.042

*The boldface frequencies are ones in which the allele is in substantially higher frequencies in at least two of the Mexican wolf lineages than in coyotes or dogs or is unique to a Mexican wolf lineage.

TABLE 4. Genetic distances [Nei, 1978] between the Mexican wolf lineages and other taxa, based on the frequencies at 20 microsatellite loci [from García-Moreno et al., 1996; Wayne, 1995]

	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Certified	0.393	0.318	0.480	0.910	0.967
Ghost Ranch		0.154	0.695	1.213	1.097
Aragón			0.671	1.227	1.021
Gray wolf				0.502	0.575
Coyote					0.667

the other taxa, particularly the northern gray wolves, suggest that there could be important adaptive differences between the Mexican wolves and the other taxa correlated with the differences indicated by these marker molecular loci.

A unique allele is defined as an allele present in a given taxon but completely missing from a sample from another compared taxon. For example, allele 200E (Table 3) is unique in the Certified lineage (with a frequency of 0.650) when compared to either the Ghost Ranch or Aragón lineages, but not when compared to gray wolves, coyotes or dogs. Table 5 gives the average frequency (and number) of unique alleles [Hedrick, 1971] in the three Mexican wolf lineages as listed in the first column when compared to the other taxa across the table. For example, the average frequency per locus of a unique allele in the Certified lineage when compared to the Ghost Ranch lineage is 0.338, and there are 24 such alleles over the 20 microsatellite loci. In other words, on average approximately one-third of the alleles by frequency in the Certified lineage are not found in the Ghost Ranch lineage. In fact, the largest values, 0.338 and 0.336, are for the Certified lineage when compared to the Ghost Ranch and Aragón lineages, respectively, probably because of the loss of alleles in these two smaller, more inbred lineages. When the Certified lineage is compared to gray wolves, the frequency of unique alleles is only 0.056. This is probably due partly to the larger sample in the gray wolves and partly to the loss of alleles in the Certified lineage because it was started with only three founders. On the other hand, when the Certified lineage is compared to coyotes and dogs, the values, 0.188 and 0.240, are fairly large, indicating that the Certified lineage has many alleles not found in these taxa.

The other comparisons between Mexican wolf lineages—that is, Ghost Ranch–Certified (0.155), Ghost Ranch–Aragón (0.140), Aragón–Certified (0.123), and Aragón–Ghost Ranch (0.097)—all have a substantial frequency of unique alleles, 10% or above, when compared individually to the other Mexican wolf lineages. In other words, both the Ghost Ranch and Aragón have unique alleles when compared to the Certified lineage, and the Ghost Ranch and Aragón have six and five alleles, respectively, that are unique to them when compared to the other lineage. There are four alleles that are unique to the Ghost Ranch and Aragón lineages when compared to the Certified lineage, 173F, 200K, 377B, and 383F (see Table 3), and they have an average frequency of 0.596 over the Ghost Ranch and Aragón lineages.

If a Mexican wolf lineage contained an allele that was found only in coyotes or dogs, then it is possible that there is some ancestry from coyotes or dogs in that lineage. There are 35 alleles that are unique to coyotes (not in gray wolves or dogs) with an average frequency of 0.106 per locus and 15 alleles unique to dogs (not in

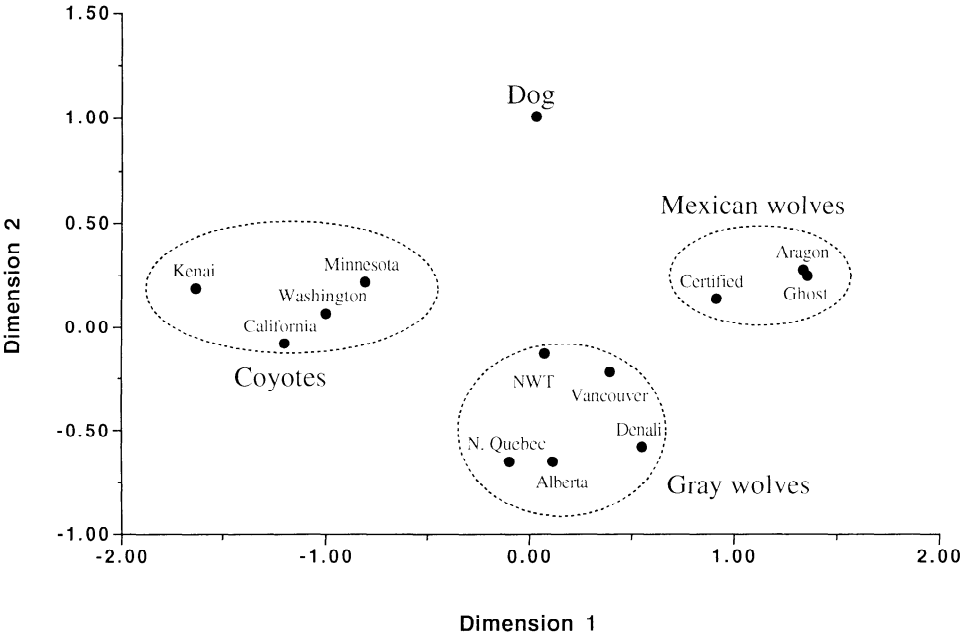


Fig. 4. Multidimensional scaling of allele frequency data of 20 microsatellite loci. Coyote and gray wolf populations are indicated by state or province. Broken ellipses encompass all taxa of a given type (e.g., Mexican wolves, gray wolves, or coyotes).

TABLE 5. Average frequency of alleles per locus (and total number of alleles in parentheses) unique to the three Mexican wolf lineages in the leftmost column when compared to the taxa across the top row for 20 loci

	Certified	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Certified	—	0.338 (24)	0.336 (23)	0.056 (5)	0.188 (9)	0.240 (16)
Ghost Ranch	0.155 (6)	—	0.140 (6)	0.002 (1)	0.188 (6)	0.198 (7)
Aragón	0.123 (4)	0.097 (5)	—	0.020 (1)	0.175 (6)	0.171 (7)

gray wolves or coyotes) with an average frequency per locus of 0.050. Only one of the unique coyote alleles, 213E, was found in the Mexican wolves, and that was only in the Certified lineage at a frequency of 0.075, as a heterozygote in individuals 23, 33, and 39. In fact, this allele is rarer in coyotes, with a frequency of only 0.017. All of these individuals were progeny of the mating between 10 and 14 for which we do not have genotypes. Because 5 was homozygous for 213L and all six of her offspring that were genotyped were homozygous for 213L, either 10 or 14 received the 213E allele from the father or perhaps, because of the relatively high mutation rate for microsatellite loci, a new mutant occurred in 10 or 14. If this later case is true, then allele 213E would have arisen independently in the Certified lineage from allele 213E in coyotes, and its presence in the Certified lineage does not suggest that there was ancestry from coyotes.

Only one of the unique dog alleles was found in any of the three Mexican wolf lineages. Allele 431B has a frequency of 0.563 in dogs and has a frequency of 0.100

in the Certified lineage, present twice in heterozygous 43 and his heterozygous mother, 13. The parents of 13 are 11 and 5, who do not carry the 431B allele. Therefore, the allele appeared to have been introduced into the lineage by 11, a male that was phenotypically a wolf and captured wild about 80 km southeast of Chihuahua City, Mexico. Again, this single allele could have arisen by mutation in 11 or 13, given the high mutation rate for microsatellite loci, could be the result of interbreeding of dogs and Mexican wolves ancestrally to 11, or could be a primitive retention of a genetic variant from the common ancestor of dogs and Mexican wolves [García-Moreno et al., 1996]. In any case, a single such allele in very low frequency suggests that if it were indicative of ancestry from dogs this ancestry represents only a very small proportion and of little biological significance.

Another way to determine if there could be ancestry from coyotes or dogs in the Mexican wolf lineages is to calculate the probability of loss of an allele from dogs or coyotes, given that one of the founders of the lineage was a wolf-dog or a wolf-coyote hybrid. For example, the initial frequency in a lineage would be, for a completely diagnostic allele (one with no sharing in frequency between Mexican wolves and coyotes or dogs), $1/2N_f$ where N_f is the number of founders in a lineage and one of these founders is a wolf-dog or wolf-coyote hybrid. For example, with two founders, the initial frequency of alleles at locus 172 that were not G would be 0.25. The probability that this allele would still be present in at least one of N progeny produced from this mating is $1 - 0.5^N$. For example, if two progeny are produced, then one or more progeny would be expected to possess the diagnostic allele 75% of the time. If there are several generations of low progeny numbers, such as in the Ghost Ranch lineage, then the probability of loss would be much higher. However, given that there is more than one diagnostic locus, then the probability that one diagnostic allele would still be present in at least one progeny would be $1 - 0.5^{NM}$ where M is the number of loci. For example, if loci 172, 200, 204, and 377 (four loci) are considered diagnostic for coyote or dog alleles in the Ghost Ranch lineage, then with two progeny the probability that we would see one of these alleles if indeed one ancestor is a wolf-dog hybrid would be $1 - 0.5^{(2)(4)}$ or 99.6%.

However, the expected contribution of male 9100 of the Ghost Ranch lineage is only 0.25 because his son was mated back to female founder 9101. Further, if 9100 was a hybrid wolf-dog, then only half his 0.25 genetic contribution would be dog (or 12.5% for the lineage). In this case, the appropriate expression for the progeny of 9102 and 9101 is $1 - 0.875^{NM}$. In other words, with two progeny one or more would be expected to possess the diagnostic allele 23.4% of the time, and with four loci we would see one of the alleles 65.6% of the time. In other words, there is still a substantial probability that a dog or coyote allele (or alleles) would have been observed if indeed one of the founders was a wolf-dog or wolf-coyote hybrid. If the Ghost Ranch male were a wolf-dog hybrid, then the initial frequency of non-Mexican wolf alleles would be 0.125, and, although 87.5% of these alleles would be expected to be reduced in frequency by genetic drift over time, 12.5% would be expected to increase. Of course, for the Ghost Ranch lineage, no unique dog or coyote alleles were observed for any of the 20 microsatellite loci.

Further, the high frequency of shared alleles in the three Mexican wolf lineages for a number of different microsatellite loci strongly argues against any dog or wolf ancestry in these lineages. For example, if one of the founders of the Ghost Ranch lineage had been a wolf-dog hybrid, then it is extremely unlikely that the Ghost Ranch

lineage would have a genetic distance of only 0.154 when compared to the Aragón lineage or would have high frequencies of so many alleles that appear to be typical of the Mexican wolf (boldface in Table 3).

Numbers of Founders in the Certified Lineage

As discussed above, it has been assumed that there were four founders of the Certified lineage, males 2 and 11, female 5, and her uncaptured mate P5 (5 was pregnant at the time of her capture) [Siminski, 1993]. However, there has always been the thought that there may only be three independent founders based on some circumstantial evidence [Siminski, 1991]. For example, male 2 was captured in Durango in 1977 and female 5 at the same location the following year. Their estimated ages at time of capture were 6 months (2) and 6 years (5). It appears that 2 was a son of 5, which would result in only three founders for the certified lineage rather than four (the three-founder scenario assumes that P5 is the father of 2 as well as the litter produced by 5 after her capture).

The multilocus DNA fingerprint data are generally consistent with the three-founder scenario [Fain et al., 1995]. Because the degree of relatedness is the same between a parent and offspring or between full sibs, the degree of similarity between 5 and 2 can be compared to that of known full sibs in the pedigree. For example, the degree of similarity between 2 and 5 (0.90) does not appear to differ from comparisons between the four animals analyzed from the known sibship from 5 and P5 (7, 8, 9, and 10), which had similarities to each other ranging from 0.80 to 0.91. Further, if 2 is compared to these four animals, its similarity ranges from 0.67–0.97, values similar to that of the known full sibs and suggesting that it might also be a full sib from the same parents. However, the average for all the wolves in the Certified lineage was also quite high at 0.80. Support for the three-founder scenario from DNA fingerprint data would be much stronger if the average similarity between unrelated Mexican wolves from the same geographic area were known, but such data are not available.

Wayne [1995] has examined whether it is possible from microsatellite loci to determine if 2 could be excluded as the son of 5. For the 16 loci polymorphic in the Certified lineage examined in these two individuals, there are no loci that could definitively support the four-founder scenario (i.e., exclude the three-founder scenario) (Table 6). In other words, for each of the 16 loci, there is at least one allele shared between 5 and 2, consistent with the hypothesis that 2 is an offspring of 5. Only a locus which had two alleles in 2 that are not present in 5 (no sharing of alleles) could be used to definitively support that another individual besides 5 is the mother of 2. It is theoretically possible to exclude the three-founder scenario if it is not true, but it is not possible to exclude the four-founder scenario.

Also, Table 6 gives the genotypes for the three known offspring of 5 (7, 8, and 9). From the genotype of 5 and these genotypes, we can construct the genotype of P5, the male parent of the offspring (right column of Table 6). We can then determine if the genotype of 2 is consistent with these two parental genotypes. For example, at locus 204, 5 is DD and P5 is AE, making the observed DE for 2 consistent. For all 16 loci, the observed 2 genotype is consistent with these parental genotypes. Looking at these data in slightly different way, 2 has two alleles found in the three known offspring of 5 (7, 8, and 9) for 15 out of the 16 loci.

TABLE 6. Genotypes for the 16 loci polymorphic in the Certified lineage that were scored in both 5 (the founding female) and 2 (the young male caught at the same location) (two loci polymorphic in the Certified lineage, 366 and 383, were not scored in both these animals) and the alleles shared for the two individuals, plus the genotypes for the known progeny of 5 (7, 8, and 9) and the genotype for the male, P5, that fertilized 5 inferred from the genotypes of 5, 7, 8, and 9

Locus	5	2	Shared	7, 8, and 9	P5
109	BB	BF	B	BF, BF, BB	BF
123	EE	EH	E	EH, EH, EH	H-
172	GG	GH	G	GH, GI, GI	HI
173	DE	DD	D	DD, —, —	D-
200	EF	EF	E, F	EE, EE, EF	E-
204	DD	DE	D	AD, AD, DE	AE
213	LL	LL	L, L	LL, LL, LL	L-
225	CC	CC	C, C	CC, CC, CC	C-
250	EE	EG	E	EG, EG, EG	G-
344	BB	BB	B, B	BB, BB, BB	B-
377	LR	LS	L	LS, LR, LL	LS
410	DD	DF	D	DD, DD, DD	D-
431	IM	IM	I, M	MM, IM, IM	M-
442	BB	BB	B, B	BB, BB, BB	B-
453	AJ	IJ	J	IJ, AI, IJ	I-
606	AB	AB	A, B	AB, AB, AA	A-

Further, using the data for the microsatellite loci, we can quantify the relationship between pairs of individuals from the index of relatedness

$$R^* = \frac{\sum \sum (P_y - P^*)}{\sum \sum (P_x - P^*)}$$

[Queller and Goodnight, 1989] where P^* is the population frequency of a given allele, excluding the compared individuals, P_x is the allele frequency in one individual, and P_y is the allele frequency in the other individual. The frequency of a given allele in an individual is 0.0, 0.5, or 1.0, depending on whether the individual is homozygous for other alleles, heterozygous for the given allele, or homozygous for the given allele, respectively. The equation is summed over all loci and all alleles at each locus. Overall, the measure varies between -1 and 1 with, for example, the expected theoretical value for comparisons between a parent and offspring or full sibs equal to 0.5 [Queller and Goodnight, 1989].

Because there are so few founders in the Certified line, many of the individuals (with the exception of the early individuals in the pedigree) share alleles from founders. As a result of this high background of sharing, the value of R^* for individuals with known relatedness of 0.5 (full-sib or parent-offspring pairs) is only 0.189 ± 0.213 ($N = 49$). Notice also that the standard deviation is larger than the mean, indicating the great variation over comparisons.

There are two comparisons using this measure that will help distinguish between the three- and four-founder scenarios: 1) in the three-founder scenario 5 and 2 are

TABLE 7. Comparison of the expected relatedness between individuals x and y under the three-founder and four-founder scenarios as compared to that observed from the microsatellite data*

Individual		Expected		Observed
x	y	Three-founder	Four-founder	
5	2	0.5	0.0	0.122
5	7	0.5	0.5	0.227
5	8	0.5	0.5	0.411
5	9	0.5	0.5	0.472
2	7	0.5	0.0	0.509
2	8	0.5	0.0	0.321
2	9	0.5	0.0	0.366
7	8	0.5	0.5	0.583
7	9	0.5	0.5	0.410
8	9	0.5	0.5	0.362

*Under the three-founder scenario, the top four pairs are between mother and offspring, and the bottom six pairs are between full sibs.

parent and offspring (as are 5 with 7, 8, and 9), and in the four-founder scenario they are unrelated (top part of Table 7), and 2) in the three founder scenario 2 is a full sib of 7, 8, and 9, while under the four-founder scenario they are unrelated (bottom part of Table 7). In the rightmost column of Table 7, the observed R^* values are given. Notice in the top part of the table there is slight reduction in the R^* value for the 5 and 2 comparison, but that it is still positive and only slightly lower than the mean given above. Most convincing are the nearly equivalent values for the known comparisons between sibs (last three rows with a mean of 0.452) and the putative sib comparisons (2 with 7, 8, and 9 with a mean of 0.399). The obvious interpretation is that these pairs are not unrelated but are closely related and that 5 is the mother of 2 and that 2 is the brother of 7, 8, and 9.

Finally, 2 and 5 had three litters with a total number of eight offspring. Two of the three males pups from this mating had abnormally developed testicles [Siminski, personal communication]. Abnormal male reproductive development can be the result of inbreeding depression, and this observation is consistent with the hypothesis that 5 and 2 were mother and son.

Overall, these findings strongly suggest that there were three founders to the Certified Mexican wolf lineage, with 2 being the son of founders 5 and P5. Although there is some small possibility that 2 could have been the son of some other unknown (related) wolves, this likelihood seems remote. To assume that there were four founders when the evidence points to only three could further jeopardize the Certified lineage by breeding related individuals. As a result, the Mexican Wolf Species Survival Group in July, 1995, officially declared the existence of three founders for the Certified lineage in future considerations.

DISCUSSION AND CONCLUSIONS

Genetic Evaluation of the Three Lineages

The molecular genetic information is consistent with the wolves in the Certified, Ghost Ranch, and Aragón lineages being Mexican wolves, *Canis lupus baileyi*. The

most definitive data are those from the microsatellite analysis which show the three Mexican wolf lineages clustering together and substantially different from northern gray wolves, coyotes, and dogs. Ten of the 20 loci have alleles that are diagnostic for Mexican wolves (namely, alleles 109C, 123H, 172G, 213L, 225C, 250G, 253D, 383G, 431I, and 442B) are all in substantially higher frequency in the three Mexican wolf lineages than in gray wolves, coyotes, or dogs.

Further, the genetic results are consistent with no past introgression from dogs and coyotes. For example, the probability that a founder of the Ghost Ranch lineage was a wolf-dog hybrid and that all the diagnostic dog microsatellite alleles have been lost from the present sample is small. Of course, in this case, the putative dog ancestry would have been quite low, at only 12.5%. The probability that the Aragón lineage had ancestry from either dogs or coyotes is also very small. On similar grounds, it is unlikely that there is recent ancestry from other subspecies of gray wolves, although it is probable that there was some historical exchange with contiguous subspecies, as occurs between most adjacent subspecies. Nowak [personal communication] notes that certain of the morphological features of a few of the skulls of captive individuals give cause for concern about the possibility of hybridization and that a more thorough analysis of the effects of captive rearing would be advisable before this possibility is eliminated. However, unlike morphology, which can be influenced by environmental factors such as captive rearing, the genetic data from the microsatellite survey cannot be influenced by the environment and are completely consistent with a conclusion of no ancestry from other species in the three lineages.

The molecular genetic data suggest that the three Mexican wolf lineages are more closely related to each other than they are to an array of northern gray wolf populations which are the geographically closest extant subspecies. No comparison was done for other subspecies because they are assumed to be more distantly related.

Combining the Three Mexican Wolf Lineages

In the following discussion, it is assumed that the most appropriate approach for combining the three lineages is to cross animals from the Ghost Ranch and Aragón lineages into the Certified lineage. Other approaches, such as introducing Certified animals into the Ghost Ranch and Aragón lineages, were considered, but were thought to be of secondary importance because wolves from the Certified lineage are designated to be used in the reintroduction of Mexican wolves into their historic range [US Fish and Wildlife Service, 1995].

The introduction of new founders to a captive breeding population is an approach that has been advocated to counter the effects of inbreeding depression and loss of genetic variation [e.g., Ballou, 1992]. The importance of the introduction of new founders depends primarily upon the initial numbers of founders and degree of inbreeding in the present captive population. For example, if the initial number of founders is quite low, as in the Certified lineage, then new founders are quite valuable to the breeding program. In this case, there are general recommendations for the incorporation of new founders into a captive breeding population [e.g., Jones et al., 1985; Odum, 1994]. However, unlike many other situations in which new individuals to be introduced into a captive breeding population are wild-caught individuals, the animals to be introduced into the Certified lineage from the Ghost Ranch and Aragón lineages have a history of inbreeding. As a result, the new founders added to the Certified lineage by combining the Ghost Ranch and Aragón animals into the Certi-

TABLE 8. Comparison among the Mexican wolf lines for measures of genetic diversity (the upper part of the table is from the microsatellite data in this report, and the lower part is from pedigree analysis)*

	Certified	Ghost Ranch	Aragón
Number of alleles	2.50	1.55	1.50
Proportion of loci polymorphic	0.90	0.45	0.45
Heterozygosity (observed, expected)	0.457, 0.403	0.128, 0.174	0.255, 0.211
Average frequency unique alleles (number)	0.337 (23.5)	0.148 (6.0)	0.110 (4.5)
Average genetic distance	0.356	0.274	0.236
Number of founders	3	2	2
Number alive (7/94)	91	18	9
Number alleles surviving	5.41	2.02	3.44
Inbreeding coefficient	0.184	0.608	0.263 (0.200 ^a)

*The average frequency of unique alleles and average genetic distance in a given lineage is the average from Tables 4 and 5 when compared to the two other lineages.

^aThe inbreeding coefficient when 7 is the mother of the litter with individuals 1 to 6.

fied lineage do not have as much genetic value as would unrelated wild-caught animals. However, the genetic history as reflected in the pedigrees in Figures 2 and 3 can be evaluated if the known pedigrees of these lineages are included in the new combined lineage and are used in future plans for matings.

As background for suggesting the proportion of representation from the three lineages in the combined lineage, both molecular and other data are relevant. There are a number of genetic and other factors that need to be considered in coming to a recommendation on this question. The top of Table 8 summarizes the relevant molecular genetic data, while the bottom of the table is concerned with other genetic considerations. For example, based on measures of genetic variation derived from microsatellite loci (the observed number of alleles, proportion of polymorphic loci, and level of heterozygosity), it appears that the Certified lineage has the most genetic variation, while the Ghost Ranch and Aragón lineages have somewhat less. Both the Ghost Ranch and Aragón lineages have only a few alleles not found in the Certified lineage, although these are in high frequency. There is a substantial genetic distance between the lineages, comparable to the size of the genetic distance between nearby populations in northern gray wolves [Roy et al., 1994].

In consideration of the other factors given in the bottom of Table 8, the Certified lineage has the largest number of founders and largest number of alleles surviving. Even assuming three founders for this lineage, the number of alleles surviving is approximately the same as the other two lineages combined. Further, the inbreeding coefficient for the Certified lineage is much lower than the Ghost Ranch lineage and probably much lower than for the Aragón lineage (remember that the value given in Table 8 is probably an underestimate because the early history of this lineage is not known). Three other factors are also of significance in giving more importance to the Certified lineage, namely, it has the best documented history, it has the greatest number of living individuals, and it is the designated lineage for use in the reintroduction. However, there is no evidence to suggest that the other two lineages are not Mexican wolves, and they contain genetic variation not present in the Certified lineage; that is, they would add descendants from at least four additional founders (two from the Ghost Ranch lineage and two or possibly three from the Aragón lineage) which were captured from different areas than the Certified lineage founders.

Although there has as yet been no documentation of inbreeding depression in the Certified lineage [Miller and Hedrick, unpublished], it is likely that there has been random fixation of some alleles that reduce fitness. Because of the higher inbreeding coefficients in the Ghost Ranch and Aragón lineages, the probability of fixation of detrimental alleles would be even higher in these two lineages. The alleles randomly fixed by inbreeding and genetic drift in the three lineages would most likely be different [e.g., Hedrick, 1985]. Therefore, crossing animals from the Ghost Ranch and Aragón lineages into the Certified lineage should introduce normal alleles at loci that may have become fixed for detrimental alleles by chance, thereby allowing selection to act against the detrimental alleles and subsequently increasing the mean fitness of the Certified lineage. The introduction of Ghost Ranch and Aragón animals may also result in some heterotic effect in the first generation of progeny resulting from these crosses.

Perhaps some guidance about the level of gene flow to recommend can come from another endangered taxon, the Florida panther. To overcome low fitness in the Florida panther, an initial level of 20% gene flow from Texas cougars has been suggested [Seal, 1994]. This level of gene flow has been shown theoretically, using population genetic models, to both result in loss of detrimental genetic variation causing low fitness and to allow retention of adaptive genetic variants [Hedrick, 1995]. Because we are suggesting ancestry or gene flow from two other lineages, 10% from the Ghost Ranch lineage and 10% from the Aragón lineage are reasonable initial values.

As a result of the findings and conclusions discussed above, there a number of recommendations that are appropriate. Wolves from all three lineages should be considered as part of the Certified lineage. Matings between the present lineages (i.e., between the Certified and Ghost Ranch and between Certified and Aragón but not between Ghost Ranch and Aragón) should be initiated as soon as possible. By merging the three lineages, the total number of founders could be increased and, with proper management, the inbreeding coefficient and mean kinship kept at a low level.

Future matings should be designed so that animals would be mated between lineages as much as feasible with the immediate goal that the ancestry of future progeny should be approximately 80% Certified lineage, 10% Ghost Ranch lineage, and 10% Aragón lineage. We feel that 10% from each of the Ghost Ranch and Aragón lineages is the minimum percentage to make a significant contribution. Because there are many more wolves in the Certified lineage than the other lineages, it may take several generations to increase the percentage of Ghost Ranch and Aragón to 10%. During this process, the success of matings within and between lineages should be monitored to determine the influence on factors such as morphology, behavior, physiology, juvenile survival, etc.

Before the percentage from the Ghost Ranch and Aragón lineages is increased significantly above 10%, all aspects of the program to combine the lineages should be carefully evaluated. In any case, the upper limit from the Ghost Ranch and Aragón lineages should not exceed 25% each. This determination is based primarily on the knowledge that the Certified lineage has been genetically well managed and has a thoroughly documented history, compared to the Ghost Ranch and Aragón lineages, and the Certified lineage should not be a minority in the overall ancestry in the captive Mexican wolves.

The recommendations outlined above for combining the three lineages were

made to the Mexican Wolf Species Survival Group in July, 1995, at its annual meeting. The group to combine the three lineages as recommended and planned six initial matings for fall and winter, 1995, between Certified and Ghost Ranch wolves or between Certified and Aragón wolves to begin the process of merging the lineages. The lineage referred to as Certified lineage in this report is now called the McBride lineage after Roy McBride, who captured its founders. Individuals in the Ghost Ranch and Aragón lineages have now been given new numerical designations in the combined captive lineage.

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