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**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered
Species**

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**Genetic Evaluation of the Three Captive Mexican Wolf
Lineages and Consequent Recommendations**

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**This report and its recommendations represent the consensus opinion of the
Genetics Committee of the Mexican Wolf Recovery Team.**

Introduction

The Genetics Committee of the Mexican Wolf Recovery Team, composed of four panel members: Mary Ashley (University of Illinois at Chicago), Philip Hedrick (Arizona State University), Gerardo Lopez Islas (Parque Zoologico de San Juan de Aragón), and Ronald Nowak (U.S. Fish and Wildlife Service) met with two technical consultants, Steven Fain (National Fish and Wildlife Forensics Laboratory) and Robert Wayne (University of California at Los Angeles), a facilitator, Steven Chambers (U.S. Fish and Wildlife Service), and the Mexican Wolf Recovery Team Leader (David Parsons, (U.S. Fish and Wildlife Service) in Albuquerque, New Mexico on July 11-12, 1994. The committee was asked by David Parsons to respond to the five specific questions given below concerning the three captive lineages of Mexican wolves, based on the genetic information prepared by the technical consultants and other background information. In addition, two other questions pertaining to the genetics of Mexican wolves were discussed and have been added as questions 6 and 7 below. Philip Hedrick was asked by David Parsons to prepare this report for the Genetics Committee summarizing the findings of the previous genetic research, the new results from the technical consultants, and giving the responses from the Genetics Committee to the questions below as well as the reasoning behind these responses.

- (1) Are wolves in the "Certified" lineage pure *Canis lupus baileyi*?***
- (2) Are wolves in the "Ghost Ranch" (a.k.a., Arizona-Sonora Desert Museum / Ghost Ranch or ASDM / GR) lineage pure *Canis lupus baileyi*?***
- (3) Are wolves in the "Aragón" lineage pure *Canis lupus baileyi*?***
- (4) Can introgression of genetic material from other canid species or other wolf subspecies be ruled out by existing data for any of the three lineages?***
- (5) What is the relationship of *Canis lupus baileyi* to other subspecies of *Canis lupus*?***
- (6) Are there three or four founders to the Certified lineage?***
- (7) Given that the three lineages are all found to be *Canis lupus baileyi*, how could they best be combined?***

Before giving responses to these questions, based primarily on molecular genetic information, first a brief history of the three captive lineages of putative Mexican wolves and the current pedigrees of each will be given, the research on morphological analysis will be summarized, and the results of the molecular genetics research will be discussed. This presentation will concentrate on aspects that are important in responding to the above questions. At the Albuquerque meeting, Steven Fain presented his results from mitochondrial DNA and multilocus DNA fingerprint analyses (Fain et al., 1995) and Robert Wayne presented results from his analysis of microsatellite loci (García-Moreno et al., 1995).

Captive Lineages of Putative Mexican Wolves

Certified Lineage

The Certified lineage consists of wolves whose lineage can be traced to wild-caught Mexican stock (USFWS 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 to 1980 and placed in captivity in the United States (Siminski, 1993). 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 #s 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, #2 appeared to be approximately six months old and was not thought to be of reproductive age. No wolves have been removed from the wild since 1980. The Certified lineage is professionally managed for maximum retention of genetic variation and founder representation (American Zoo and Aquarium Association, 1994) and has been since its inception.

Figure 1 gives the current pedigree 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 #2 and #5 leads to their progeny #16, #17, etc. (see the figure legend for information about the symbols used). As of July, 1994, the Certified population numbered 92 living animals (indicated by closed symbols in Figure 1), 79 in the United States and 13 in Mexico. From analysis of this pedigree, the average inbreeding coefficient of the living wolves is 0.106 (this and other values for the three lineages are summarized in Table 6). In addition, if we assume that all the alleles in the founders were different at a given locus (eight alleles in the four founders), then given the structure of the pedigree in Figure 1, it is expected that on average 7.03 alleles (or 87.8% of the original alleles) would be remaining in the living population. We will discuss below the possibility that there were three, not four, founders of the Certified lineage. If there are only three founders (#2 is the son of #5 and #P5 as are #7, #8, etc.), then the average inbreeding coefficient is much higher at 0.184 and the expected number of alleles surviving out of the original six alleles is 5.41 (or 90.2% of the original number).

Ghost Ranch Lineage

The Ghost Ranch lineage is all presently in the United States and descends from two founders. The founding male was trapped alive in 1959 in the Tumacacori Mountains of southern Arizona, approximately ten 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 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 is not available.

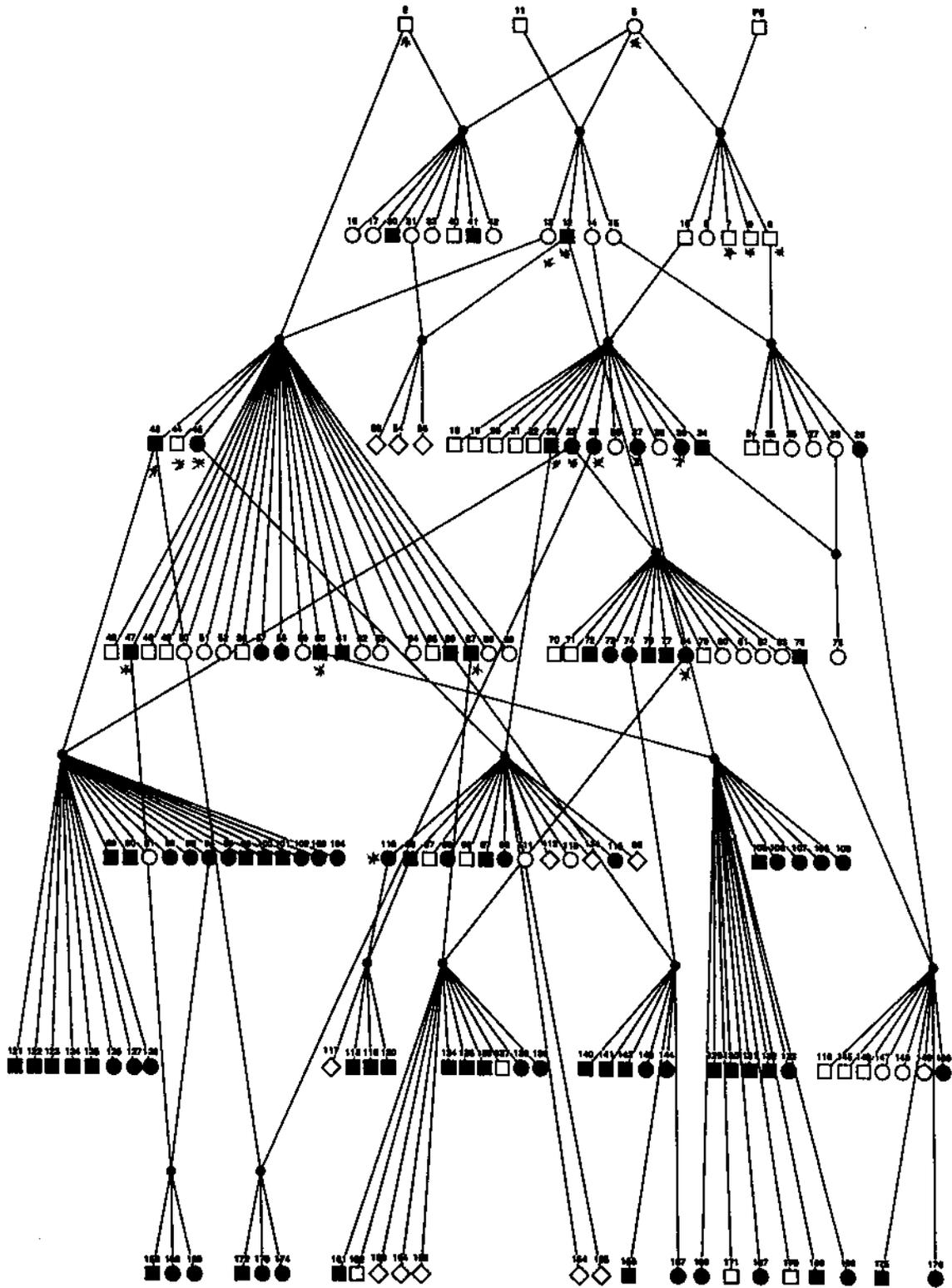


Figure 1. The pedigree of the Certified lineage as of July, 1994, where squares, circles, and diamonds, indicate males, females, and wolves of unknown sex, respectively. Darkly shaded and open symbols indicate living and dead wolves, respectively. The wolves sampled by García-Moreno et al. (1995) for microsatellite variation are indicated by asterisks.

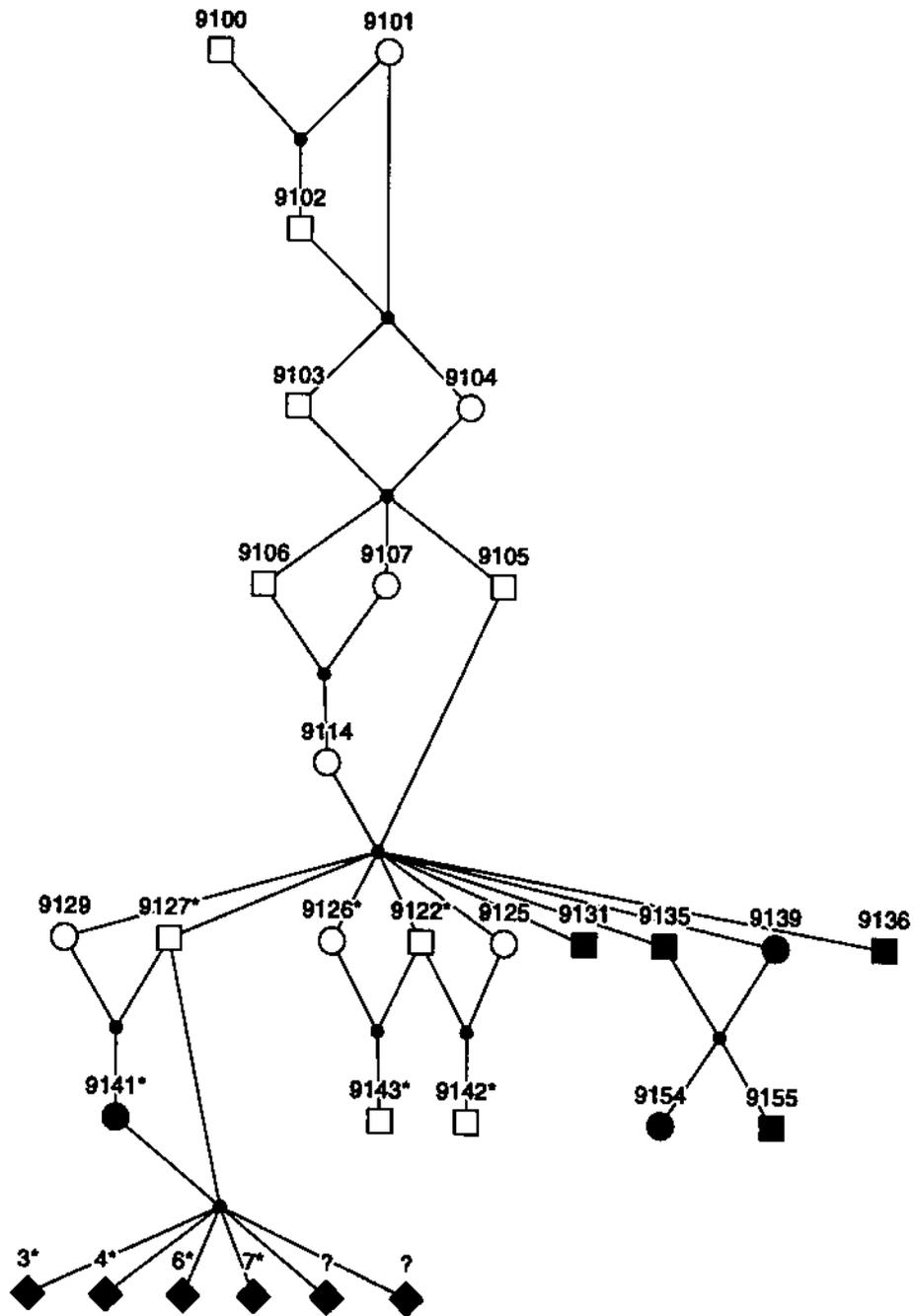


Figure 2. The pedigree of the Ghost Ranch lineage where squares, circles, and diamonds, indicate males, females, and wolves of unknown sex, respectively. Darkly shaded and open symbols indicate living and dead wolves, respectively. The wolves sampled by Garcia-Moreno et al. (1995) for microsatellite variation are indicated by asterisks.

The pedigree for this lineage is given in Figure 2 using the same symbols as in Figure 1. Eighteen animals from this lineage are alive, thirteen of which are indicated in Figure 2 (Siminski, personal communication). The inbreeding coefficient in the living individuals is quite high, approximately 0.608. If we assume that there were four alleles in the founders, only 2.02 alleles (or 50.5% of the original number) are surviving in the living animals.

Aragón Lineage

This lineage has been maintained at the San Juan de Aragón Zoo in Mexico City since 1965 and are all presently in Mexico. Founders of this lineage 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 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 Aragon group in 1987. No subsequent interbreeding of the hybrids and Aragón group occurred. Examination of cranial morphology, reproductive chronology, behavior, and allozyme composition provide no evidence to indicate hybridism in the Aragón lineage (Lopez and Vazquez, 1991).

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 is 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 number of alleles surviving from the initial four is 3.44 or 86% of the initial number. In Figure 3, it is assumed 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. In Figure 3, it is also assumed that #-02 is the mother of both #-23 and #8. It is possible that another female founder, #-03, was the mother of #7 and #8, but there is no information to support one alternative over the other. If a third founder was the mother of #8, then the inbreeding coefficient and the number of surviving alleles would be higher than given above.

Morphological Analyses

Bogan and Mehlhop (1983) compared 25 skull measurements among five subspecies of gray wolves from southwestern North America, dogs, and coyotes. Among the skulls examined were eight specimens from the captive 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 Mehlhop (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. monstrabilis*, which had ranges adjacent and mainly to the north of *baileyi*, be included

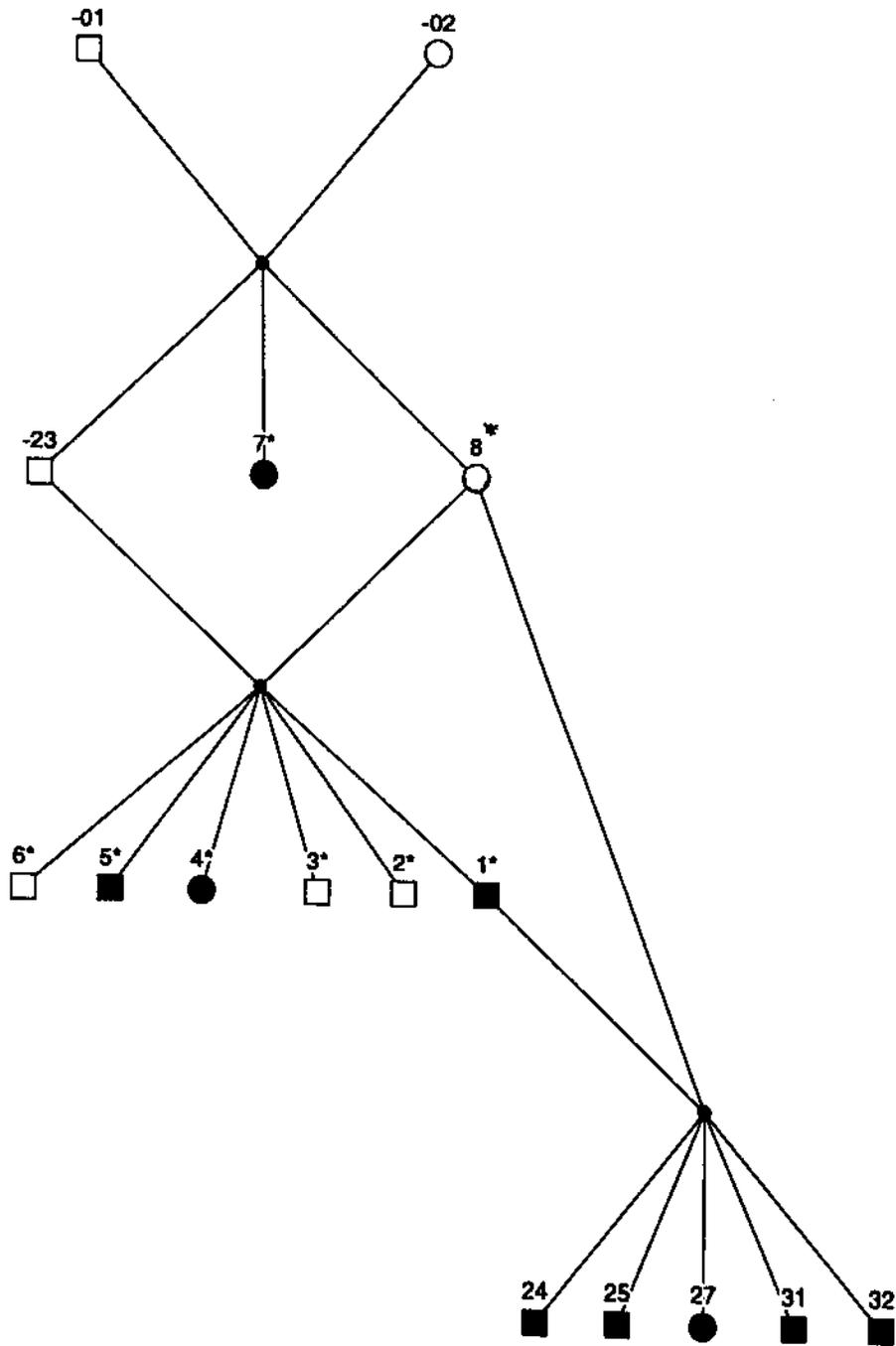


Figure 3. The pedigree of the Aragón lineage where squares, circles, and diamonds, indicate males, females, and wolves of unknown sex, respectively. Darkly shaded and open symbols indicate living and dead wolves, respectively. The wolves sampled by García-Moreno et al. (1995) for microsatellite variation are indicated by asterisks.

within *C. l. baileyi*. However, morphological analyses by both Hoffmeister (1986) and Nowak (1995) indicated that *C. l. mogollonensis* and *C. l. monstrabilis* 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 Mehlhop (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 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 sample size was too small to be definitive; but that his results are, nevertheless, suggestive of the purity of the Aragón wolves.

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 but are still within the lower limits of the size range for *C. l. baileyi*. Nowak (personal communication) also had 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. A thorough examination of these effects should be undertaken to allow differentiation of the influence of captive rearing and maintenance from the effect of possible past hybridization with other species.

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, and 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 they 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 resulted in the difference between the Certified and Ghost Ranch samples at the *Got* locus. With only a few variable loci, it is only possible to suggest that the three taxa, 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 ancestry of male founders. Shields et al. (1987) and Wayne et al. (1992)

Table 1. The 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

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 Aragon 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 as stated by Wayne et al. (1992), Wayne (personal communication)) and also found that they were identical and 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 eleven 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

Table 2. The difference between the Certified Mexican wolf lineage and other taxa for mtDNA. 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 --.

	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Shields et al. (1987)	0	--	1 to 4	15 to 17	1 to 4
Wayne et al. (1992)	0.00	--	0.10 to 0.51	3.06	--
Fain et al. (1995)	0.0	1.2	0.2	8.3	1.8

wolves while the dog sequence was also somewhat different from the wolves (see Table 2).

First, 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 is from coyotes. Other conclusions are about the relationship of Aragón 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 percent 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 Certified, $N = 33$; Ghost Ranch, $N = 10$; and 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.) 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 Aragon 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 a number of advantages when compared to other genetic markers and are the loci of choice at this time to use in identifying differences between closely related taxa. The main advantages are that microsatellite loci are highly variable, fast evolving, codominant, nuclear, and can be analyzed with standard population genetic statistics because alleles and genotypes are identifiable.

García-Moreno et al. (1995) examined variation at ten microsatellite loci in a samples of Certified ($N = 21$), Ghost Ranch ($N = 10$), Aragón ($N = 8$) wolves as well as gray wolves ($N = 84$), coyotes ($N = 142$), and dogs ($N = 42$, one individual from 42 different breeds, including one Siberian husky and one Alaskan husky). Table 3 gives the frequencies of all the alleles found in the three Mexican wolf lineages for these ten microsatellite loci. First, notice that the Certified lineage is polymorphic at all ten loci while Ghost Ranch is polymorphic at only three loci and Aragón at six loci. The Certified lineage has the highest average number of alleles per locus, 2.5, and the highest average observed heterozygosity, 0.495 (see Table 6 for these values). In the other extreme, the Ghost Ranch lineage has only 1.3 alleles per locus and has a very low observed heterozygosity of 0.040. The Aragon lineage has values between the two other lineages

Table 3. Frequencies of all the alleles found in the Mexican wolf lineages (García-Moreno et al., 1995). 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.

Gene	Allele	Certified	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
109	B	0.619	0.600	0.000	0.290	0.184	0.410
	C	0.238	0.400	0.286	0.119	0.058	0.000
	F	0.143	0.000	0.714	0.091	0.141	0.244
123	E	0.524	0.100	0.500	0.535	0.052	0.149
	H	0.476	0.900	0.500	0.059	0.392	0.270
172	G	0.810	1.000	1.000	0.089	0.015	0.000
	H	0.143	0.000	0.000	0.322	0.337	0.012
	I	0.048	0.000	0.000	0.458	0.158	0.952
200	E	0.619	0.000	0.000	0.083	0.102	0.207
	F	0.381	0.000	0.357	0.157	0.107	0.195
	K	0.000	1.000	0.643	0.004	0.015	0.000
204	A	0.200	0.000	0.000	0.479	0.886	0.303
	B	0.150	0.000	0.000	0.083	0.114	0.026
	D	0.500	1.000	0.857	0.376	0.000	0.026
	E	0.150	0.000	0.143	0.054	0.000	0.000
213	E	0.071	0.000	0.000	0.000	0.040	0.000
	L	0.929	1.000	1.000	0.237	0.202	0.076
225	B	0.048	0.200	0.286	0.326	0.084	0.439
	C	0.952	0.800	0.714	0.288	0.366	0.012
250	E	0.452	0.000	0.000	0.056	0.084	0.000
	G	0.548	1.000	1.000	0.267	0.142	0.214
344	A	0.333	0.000	0.000	0.608	0.578	0.000
	B	0.667	1.000	1.000	0.233	0.359	0.878
377	B	0.000	1.000	0.857	0.102	0.025	0.025
	L	0.500	0.000	0.143	0.369	0.094	0.000
	R	0.350	0.000	0.000	0.000	0.000	0.000
	S	0.150	0.000	0.000	0.000	0.000	0.000

with 1.6 alleles per locus and an observed heterozygosity of 0.329. 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 missing in gray wolves, coyotes, and dogs. For locus 204, the two major alleles in the Mexican wolves, D and E, are missing or in low frequency in coyotes and dogs and in lower frequency in gray wolves. Allele 213L is either fixed or in very high frequency in the three Mexican wolf lineages and much lower frequency in the other taxa. 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 both the genetic distance measure of Nei (1978). Examining these values, the genetic distances are smallest among the three Mexican wolf lineages, ranging from 0.09 to 0.32. Notice that the rankings of these three values are the same as 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 value for the Certified-Ghost Ranch comparison may be in part due to the fixation of alleles at six of the ten loci in the Ghost Ranch because of its history of small population size. 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 alleles frequencies for the Mexican wolf lineages and dogs given in Table 3 and alleles frequencies for a number of populations of northern gray wolves and

Table 4. Genetic distances (Nei, 1978) between the Mexican wolf lineages and other taxa based on the frequencies at ten microsatellite loci (García-Moreno et al., 1995).

	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Certified	0.32	0.26	0.67	0.85	1.02
Ghost Ranch		0.09	1.10	1.26	1.17
Aragón			0.91	1.26	1.09
Gray wolf				0.52	0.67
Coyote					0.76

coyotes to show how these taxa separate in a multidimensional scaling analysis (Borg, 1981). Notice that in this two-dimensional representation of allele frequency variation that the Mexican wolf lineages cluster together in the lower right away from the other taxa indicating that they share the closest common ancestry and are distinct from the other taxa. The six coyote populations from throughout the United States cluster to the left, the

seven northern gray wolf populations from the United States and Canada cluster to the upper center, and the dog to upper right. The large differences here and for the genetic distances in Table 4 between the Mexican wolf lineages and the other taxa, particularly the northern gray wolves, indicates suggests that there could be important adaptive differences between the Mexican wolves and the other taxa.

Table 5 gives the average frequency (and number) of unique alleles (Hedrick, 1971) in the three Mexican wolf lineages (listed on the left hand side of the table) as compared to the other taxa along the top of the table. For example, on average over the ten loci 0.116 and 0.306 of the alleles in the Certified line are not present at all in coyotes and dogs, respectively. Only 0.058 of the alleles in the Certified lineage are unique when compared to gray wolves but 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 four (or three) founders. The largest value, 0.370, is for the Certified lineage when compared to the Ghost Ranch lineage probably because of the loss of alleles in the Ghost Ranch lineage. Note that all of the Mexican wolf lineages have a substantial frequency of unique alleles when compared individually to the other Mexican wolf lineages. However, the two alleles that are unique to the Ghost Ranch and Aragon lineages when compared to

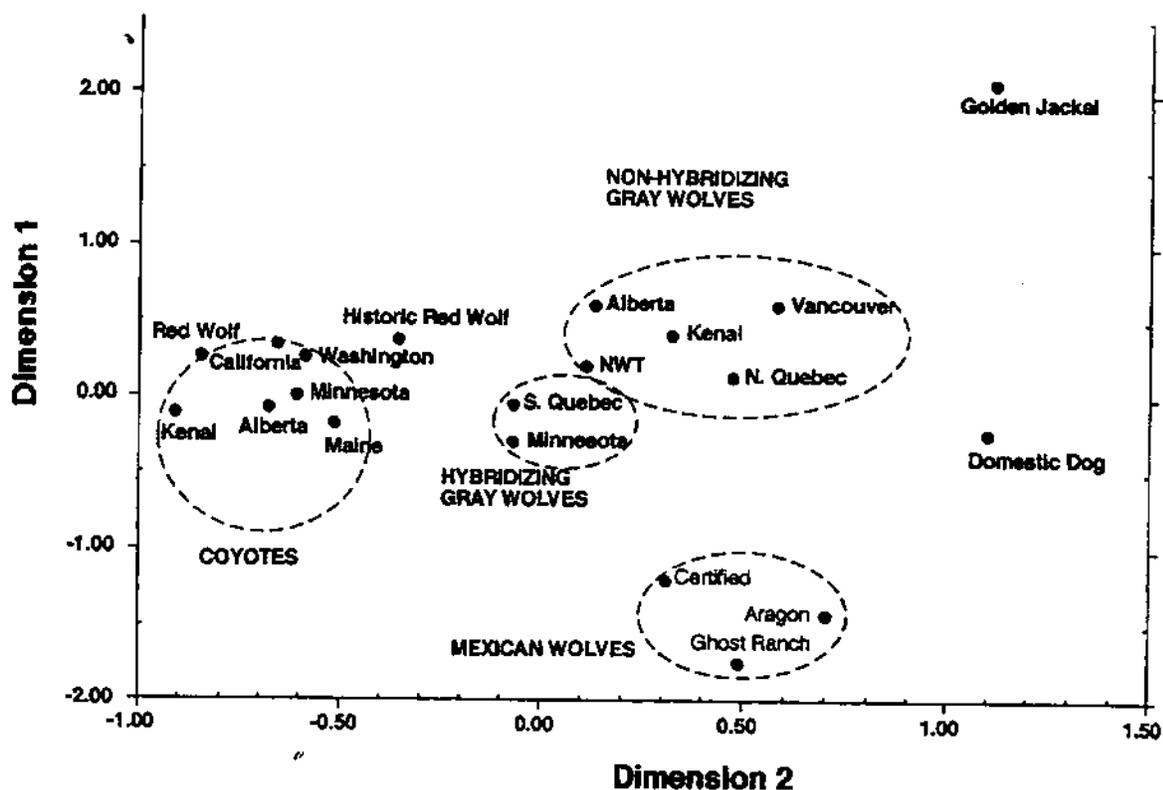


Figure 4. Multidimensional scaling of allele frequency data of ten microsatellite loci (from García-Moreno et al., 1995). Coyote and gray wolf populations are indicated by state or province.

the Certified lineage, 200K and 377B (see Table 3), are present in high frequency in both the Ghost Ranch and Aragon lineages.

If a Mexican wolf lineage contained an allele that was found only in dogs or only in coyotes, then it possible that there is some ancestry from that taxon in the lineage. There are six alleles unique to dogs (not in gray wolves or coyotes) with an average frequency per locus of 0.024 and 11 alleles that are unique to coyotes (not in gray wolves or dogs) with an average frequency of 0.018 per locus. None of the unique dog alleles are found in any of the three Mexican wolf lineages. Only one of the 11 unique coyote alleles, 213E, was found in the Mexican wolves and that was only in the Certified lineage at a frequency of 0.071. Because of the relatively high mutation rate for microsatellite loci, it is possible that this allele in the Certified lineage arose independently from allele E in coyotes so that the presence of it in the Certified lineage does not necessarily suggest that there was ancestry from coyotes. In other words, there is no evidence for unique dog alleles in the Mexican wolves and only one coyote allele that may be present in one of the lineages.

A more powerful way to ask the same question 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 (no sharing in frequency) allele, $1/2N_f$ 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 allele at locus 172 that were not

Table 5. Frequency of unique alleles (number of alleles for ten loci in parentheses) in the Mexican wolf lineages as compared to the other taxa (data from García-Moreno et al., 1995).

	Certified	Ghost Ranch	Aragón	Gray wolf	Coyote	Dog
Certified	--	0.370 (14)	0.314 (11)	0.058 (3)	0.116 (4)	0.306 (9)
Ghost Ranch	0.200 (2)	--	0.060 (1)	0.000 (0)	0.100 (1)	0.240 (3)
Aragón	0.150 (2)	0.136 (4)	--	0.000 (0)	0.100 (2)	0.222 (5)

G would be 0.25. The probability that this allele would be 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 75% of the time one or more progeny should still have the diagnostic allele. However, 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, 20, 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%. If we look at this value over generations, the value

will become somewhat lower but there is still a very high probability that a dog or coyote allele (or alleles) would have been observed in substantial frequency if indeed one of the founders was a wolf-dog or wolf-coyote hybrid.

Overall then, 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 0.09 with the Aragon lineage and have high frequencies of so many alleles that appear to be typical of the Mexican wolf (boldface in Table 3). If the Ghost Ranch male was a wolf-dog hybrid, then the initial frequency of non-Mexican wolf alleles would be 0.25 and although 75% of these would be expected to be reduced in frequency by genetic, 25% would be expected to increase. From the microsatellite data, which gives reasonable power to observe such alleles, there is no indication at all of such a hybrid origin.

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). However, 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 six months (#2) and six years (#5). It is possible 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). Figure 5 gives the four-founder and three-founder pedigrees (founder #11 is not given in either pedigree for clarity). If there were only three founders, then the pedigree calculations would be significantly higher than the four-founder scenario (see above for the inbreeding coefficients and number of alleles surviving).

The multilocus DNA fingerprint data is generally consistent with 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 sibship of #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 to 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 (personal communication) has examined whether it is possible from microsatellite loci to determine if #2 could be excluded as the son of #5. For the ten loci examined, there are no loci that could definitively support the four-founder scenario, i.e., exclude the three-founder scenario (see Figure 5). Only a locus which had an allele in #2

that is not present in #5, could be used to definitively support that another individual besides #5 is the mother of #2. In other words, it may be possible to exclude the three-founder scenario with a highly variable locus but it is not possible to exclude the four-founder scenario. With more variable loci, the relative likelihood of the three-founder or four-founder scenarios could be estimated.

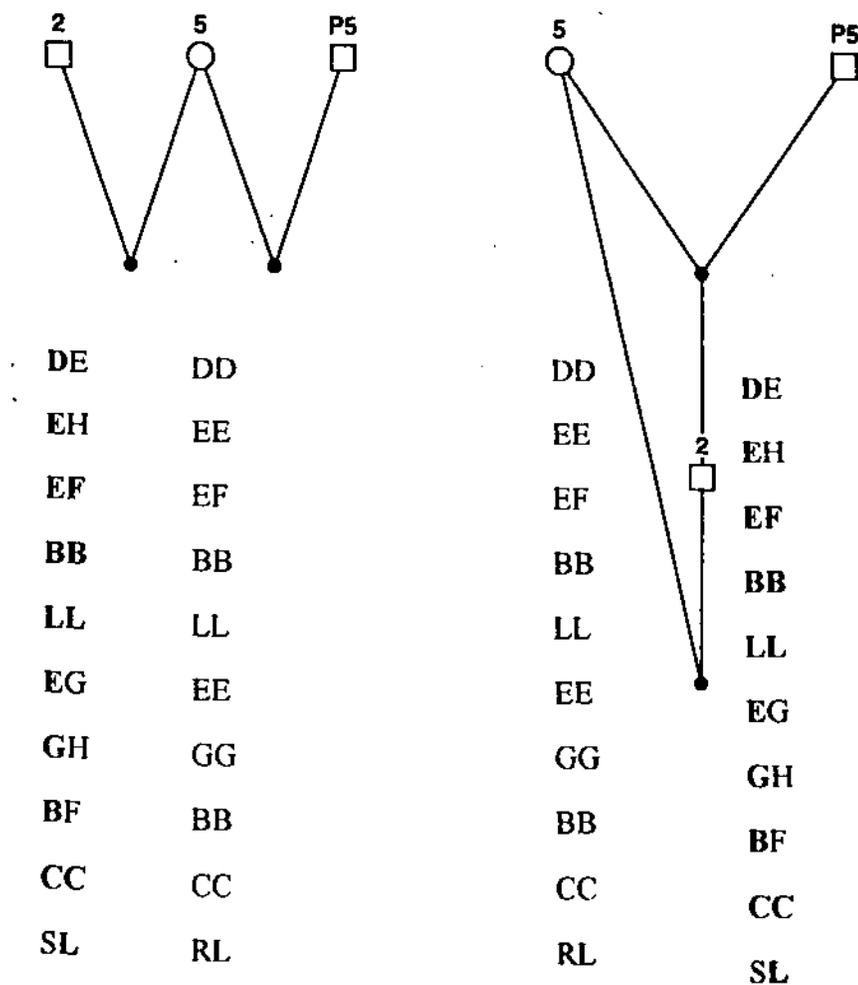


Figure 5. The four-founder and three-founder scenarios for the origination of the Certified lineage. The letters indicate the genotypes at the ten microsatellite loci for #2, the young male, and #5, the founder female and the bold-faced letters indicate alleles in #5 found in #2.

In addition, #2 and #5 had three litters with a total number of eight offspring. Four of these pups died shortly after birth and two of the surviving four had abnormally developed testicles (Fain et al., 1995). Both juvenile mortality and abnormal male reproductive development can be the result of inbreeding depression and these observations are consistent with the hypothesis that #5 and #2 were mother and son.

Combining the Three Mexican Wolf Lineages

In the following discussion, it is assumed that the most appropriate initial approach for combining the three lineages is to introduce 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 at this time because wolves from the Certified lineage are designated to be used in the reintroduction.

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 here themselves 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 Certified lineage do not have as much genetic value as would unrelated wild-caught animals.

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 6 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, the Aragón lineage somewhat less and Ghost Ranch lineage even less. In particular, the low level of heterozygosity in the Ghost Ranch lineage is significant. However, this lower level is not unexpected given the history of close inbreeding in the Ghost Ranch lineage. 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 (Wayne, personal communication).

In considering the other factors given in the bottom of Table 6, the Certified lineage has the largest number of founders and highest number of alleles surviving. Even if there are only 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 the value given in Table 6 is probably an underestimate because the early history of this lineage is not known). Three

other factors are also of significance to give more importance to the Certified lineage, namely, it has the best documented history of the lineages, has the most number of living individuals, and 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, i.e., they would add

Table 6. Comparison among the Mexican wolf lines for measures of genetic diversity (the upper part of the table is from the microsatellite data of García-Moreno et al. (1995) and the lower part from pedigree analysis).

	Certified	Ghost Ranch	Aragón
Number of alleles	2.5	1.3	1.6
Proportion of loci polymorphic	1.0	0.3	0.6
Heterozygosity (observed, expected)	0.495, 0.436	0.040, 0.103	0.329, 0.316
Average freq. unique alleles (no.)	0.310 (12.5)	0.130 (1.5)	0.143 (3.0)
Average genetic distance	0.286	0.204	0.173
Number of founders	4 (3*)	2	2
Number alive (8/94)	92	18	9
Number alleles surviving	7.03 (5.41*)	2.02	3.44
Inbreeding coefficient	0.106 (0.184*)	0.608	0.263 (0.200**)

* The values when it is assumed that there are only three founders.

** The inbreeding coefficient when #7 is the mother of the litter with individuals #1- #6.

descendants from at least four additional founders (two from the Ghost Ranch lineage and two or possibly three from the from Aragón lineage) which are from different areas from the Certified lineage to the number of founders.

Although there has been as of yet no documentation of inbreeding depression in the Certified lineage (Miller and Hedrick, in preparation), 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 different 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 and allow selection to act against the detrimental alleles and subsequently increase 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 progeny.

Perhaps some guidance about the level of gene flow to recommend can come from another endangered taxa, the Florida panther. To overcome low fitness in the Florida panther, a 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. After this level has been achieved, then a thorough evaluation of the phenotypes, including morphology, behavior, physiology, juvenile survival, and other traits deemed to be significant, of the progeny of crosses between the lineages should be undertaken. Because we wish to increase the total number of founder genomes by the introduction of the other two lineages into the Certified lineage, not a consideration in the Florida panthers, further gene flow up to a maximum of 25% from each Ghost Ranch and Aragón, could be considered after the initial combining is evaluated as suggested above.

Responses to the Questions

(1) Are wolves in the Certified lineage pure *Canis lupus baileyi*?

(2) Are wolves in the Ghost Ranch (a.k.a. Arizona-Sonora Desert Museum) lineage pure *Canis lupus baileyi*?

(3) Are wolves in the Aragón lineage pure *Canis lupus baileyi*?

Yes, the molecular genetic information is consistent with the wolves in the Certified, Ghost Ranch, and Aragón lineages being *Canis lupus baileyi*. The most definitive data is that from the microsatellite analysis which shows the three Mexican wolf lineages clustering together and substantially different from northern gray wolves, coyotes, and dogs. On a specific locus level, alleles 109C, 172G, 204D, 109C, 213L, 225C, and 250G are all in much higher frequency in the three Mexican wolf lineages than in any of the other taxa and serve as diagnostic alleles for Mexican wolves.

(4) Can introgression of genetic material from other canid species or other wolf subspecies be ruled out by existing data for any of the three lineages?

The genetic results are consistent with no introgression from dogs and coyotes. For example, the probability that a founder of the Ghost Ranch lineage was a wolf-dog hybrid and all the diagnostic dog microsatellite alleles have been lost from the present sample is very small. Also, the probability that the Aragón lineage had ancestry from either dogs or coyotes is 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 might be eliminated. However, unlike morphology, which can be influenced by the environmental factors, such as captive rearing, the genetic data from the microsatellite survey cannot be influenced by the environment and are completely consistent with no ancestry from other species in the three lineages.

(5) What is the relationship of Canis lupus baileyi to other subspecies of Canis lupus?

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

(6) Are there three or four founders to the Certified lineage?

The molecular genetic evidence at this point does not show definitively that there were four founders. In other words, it is presently consistent with either three or four founders but further microsatellite analysis may exclude the three-founder scenario or alternatively show that there is a high probability of three founders. Inbreeding coefficients, mean kinships, etc. for both the three- and four-founder scenarios should be evaluated in future breeding decisions.

(7) Given that the three lineages are all found to be Canis lupus baileyi, how could they best be combined?

First, wolves from all three lineages should be considered as part of the Certified lineage. Second, matings between the present lineages 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 is the minimum percentage to make a significant contribution. Because there are many more wolves in the Certified lineage than the other lineages and there may be some logistical problems with matings including the Aragón lineage, 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 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.

Acknowledgments

I wish to thank Phil Miller for help in the pedigree analysis and Peter Siminski for assistance in reconstructing the pedigrees for the Ghost Ranch and Aragón lineages and Gerardo Lopez for assistance in reconstructing the pedigree for the Aragón lineage. I appreciate the comments of Phil Miller and Joel Parker on an earlier draft of the report.

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**Final Progress Report (June, 1995)
Heritage Fund Project #I94038**

**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered
Species**

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I. Inbreeding Depression in the Captive Population

We have now accumulated all the data on both age and cause of death for all deceased Mexican wolves. These data are now being analyzed to determine the influence of inbreeding on longevity in captive wolves. If inbred individuals showed lower longevity, one could conclude that inbreeding depression was occurring in the captive wolf population. The results are not completed but are planned to be presented at the Mexican Wolf Species Survival Plan/Recovery Program on July 28 and 29 in Colorado Springs. The written report on inbreeding depression will be submitted to ADGF when it is complete.

II. Mexican Wolf Recovery Team

Phil Hedrick is now on the Mexican Wolf Recovery Team and is the representative to the team from the Genetics Committee of the Mexican Wolf Recovery Team. He has written a 23-page report evaluating the information from Wayne and the pedigree information using Pedpack on the Certified Lineage. Recently we have constructed pedigrees of the two other Mexican wolf lineages as part of an evaluation of those lineages before they are integrated into the Certified. This is also part of the report for the Mexican Wolf Recovery Team and a copy is included. Much of the effort in the past several months of the grant was towards preparing this report.

**Fourth Quarter Progress Report (June, 1995)
Heritage Fund Project #I94038**

**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered
Species**

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I. Inbreeding Depression in the Captive Population

We have now accumulated all the data on both age and cause of death for all deceased Mexican wolves. These data are now being analyzed to determine the influence of inbreeding on longevity in captive wolves. If inbred individuals showed lower longevity, one could conclude that inbreeding depression was occurring in the captive wolf population. The results are not completed but are planned to be presented at the Mexican Wolf Species Survival Plan/Recovery Program on July 28 and 29 in Colorado Springs. The written report on inbreeding depression will be submitted to ADGF when it is complete.

II. Mexican Wolf Recovery Team

Phil Hedrick is now on the Mexican Wolf Recovery Team and is the representative to the team from the Genetics Committee of the Mexican Wolf Recovery Team. He has written a 23-page report evaluating the information from Wayne and the pedigree information using Pedpack on the Certified Lineage. Recently we have constructed pedigrees of the two other Mexican wolf lineages as part of an evaluation of those lineages before they are integrated into the Certified. This is also part of the report for the Mexican Wolf Recovery Team and a copy is included with the final report. Much of the effort in the past several months of the grant was towards preparing this report.

**Third Quarter Progress Report (April, 1995)
Heritage Fund Project #I94038**

**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered
Species**

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I. Inbreeding Depression in the Captive Population

As in the first two quarter reports, we are accumulating data on both age and cause of death for all deceased Mexican wolves. These data would then be used in a statistical analysis of the influence of inbreeding on longevity in captive wolves. If inbred individuals showed lower longevity, one could conclude that inbreeding depression was occurring in the captive wolf population.

These data are, for the most part, compiled. Records at the Arizona-Sonora Desert Museum in Tucson AZ were used to acquire this information, with help from Mr. D. Peter Siminski, Mexican wolf studbook keeper and coordinator of the Mexican Wolf Captive Management Committee. There are 86 deaths in the studbook database as of July 7 1994, but only about 60% of those deaths are ascribed to a particular cause. We are obtaining information from other institutions by phone using an assistant in order to complete the dataset.

II. Microsatellite Data and Pedigree Analysis

As we stated in the last report, Dr. Robert Wayne of UCLA has conducted a survey of variability at a number of microsatellite loci in the captive Mexican wolf population. The pedigree analysis software PEDPACK has recently been employed to begin the analysis of data from the ten microsatellite loci Dr. Wayne has developed. We have been using the package to check for proper segregation (i.e., to determine if an individual's genotype is consistent with that of its parents') and have found some initial problems which now have been rectified.

Founder gene survival calculations (see First Quarterly Report) indicate that about seven of eight originally distinct (not identical by descent) founder genes have survived in the certified pedigreed population (assuming four founders). This represents a high degree of retention of genetic variation in this intensively managed population. The two

uncertified lines, known as the Aragon and Ghost Ranch lineages, were founded by two individual animals and have not been managed to the same degree as the certified lineage. Consequently, they have experienced higher levels of inbreeding and have presumably had a greater loss of allelic diversity compared to the certified lineage. Detailed knowledge of the degree of retention of allelic diversity through pedigree analysis and molecular genetic work will allow us to evaluate alternative strategies for the incorporation of the uncertified lineages into the managed population.

III. Mexican Wolf Recovery Team

Phil Hedrick is now on the Mexican Wolf Recovery Team and is the representative to the team from the Genetics Committee of the Mexican Wolf Recovery Team. He has written a 25-page report evaluating the information from Wayne and the pedigree information using Pedpack on the Certified Lineage. Recently we have constructed pedigrees of the two other Mexican wolf lineages as part of an evaluation of those lineages before they are integrated into the Certified. This is also part of the report for the Mexican Wolf Recovery Team and will be included in the final report for this project.

**Second Quarter Progress Report (January, 1995)
Heritage Fund Project #I94038**

**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered Species**

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I. Inbreeding Depression in the Captive Population

As of the First Quarter Report, we were accumulating data on both age and cause of death for all deceased Mexican wolves. These data would then be used in a statistical analysis of the influence of inbreeding on longevity in captive wolves. If inbred individuals showed lower longevity, one could conclude that inbreeding depression was occurring in the captive wolf population.

These data are, for the most part, compiled. Records at the Arizona-Sonora Desert Museum in Tucson AZ were used to acquire this information, with help from Mr. D. Peter Siminski, Mexican wolf studbook keeper and coordinator of the Mexican Wolf Captive Management Committee. There are 86 deaths in the studbook database as of July 7 1994, but only about 60% of those deaths are ascribed to a particular cause. No data exists in the ASDM records for the remaining deaths. We will attempt to contact those institutions that did not provide data in order to improve the dataset.

II. Microsatellite Data and Pedigree Analysis

Dr. Robert Wayne of UCLA has conducted a survey of variability at a number of microsatellite loci in the captive Mexican wolf population. Microsatellite loci consist of a variable number of repeats of short nucleotide sequences and evolve through the gain or loss of repeat units, rather than direct nucleotide substitutions. Because of their relatively high mutation rates, microsatellite loci can be used to detect differentiation among small populations that cannot be revealed by analysis of more slowly evolving loci. Dr. Wayne has used this technique to assess the genetic purity of both the certified wolf lineage as well as the two uncertified lineages.

Our collaboration with Dr. Wayne has recently expanded to include other areas of interest contained in our Heritage Fund research plan. The pedigree analysis software PEDPACK has recently been employed to begin the analysis of data from the ten microsatellite loci Dr. Wayne has developed. However, segregation analysis using the package has found errors in some of the

loci (i.e., an individual's genotype is found to be inconsistent with that of its parents'). We are currently working with Dr. Wayne to correct these recording errors before proper analysis of the genotype data is started.

Analysis of these data will allow us to address a number of issues regarding Mexican wolf management. For example, the true number of founding animals has not been resolved despite some initial molecular genetic analysis. Microsatellite loci, acting as true loci with codominant alleles, can help us finally resolve this issue. Unfortunately, we do not have sufficient numbers of loci typed in enough individuals to be able to unambiguously determine if #2 is in fact an offspring of #5 or itself an unrelated founder. Dr. Wayne is currently working on typing animals at 10 additional microsatellite loci to address this shortcoming.

Additionally, these analysis techniques will help us to more accurately compare the allele frequencies in the certified lineage with the uncertified lineages as well as with coyotes, dogs, and other gray wolves. PEDPACK analysis on microsatellite genotypes will give us insight into the most likely distribution of alleles in the founders, which can then be used to compare allele frequencies between appropriate groups.

III. Integration of Captive Wolf Lineages

Founder gene survival calculations (see First Quarterly Report) indicate that about seven of eight originally distinct (not identical by descent) founder genes have survived in the certified pedigreed population (assuming four founders). This represents a high degree of retention of genetic variation in this intensively managed population. The two uncertified lines, known as the Aragon and Ghost Ranch lineages, were founded by fewer animals and have not been managed to the same degree as the certified lineage. Consequently, they have experienced higher levels of inbreeding and have presumably had a greater loss of allelic diversity compared to the certified lineage. Detailed knowledge of the degree of retention of allelic diversity through pedigree analysis and molecular genetic work will allow us to evaluate alternative strategies for the incorporation of the uncertified lineages into the managed population. Earlier work by P. Hedrick on the genetic consequences of the proposed introduction of Texas cougars into the Florida panther wild population indicates that introgression of one or more lineages into another line can both increase heterozygosity and retain adaptive alleles in the population receiving migrants. Similar analyses are currently being planned for the integration of Mexican wolf lineages.

First Quarter Progress Report (October, 1994)
Heritage Fund Project #I94038

**Pedigree Analysis of the Global Captive Population of
the Mexican Wolf (*Canis lupus baileyi*): Genetic Management of an Endangered Species**

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I. Genetic Characterization of the Pedigreed Population

Using studbook data generously provided by Mr. D. Peter Siminski of Tucson's Arizona-Sonora Desert Museum, Mexican wolf studbook keeper and coordinator of the Mexican Wolf Captive Management Committee, I began the project by constructing a detailed pedigree of the current captive wolf population. This pedigree, with data current through 7 July 1994, is shown in Figure 1. The pedigree is shown as a marriage node graph, where offspring are separated from their parents by a large black dot or node; for example, littermates 6-10, found in the upper right-hand corner of the pedigree, were produced by the mating between founders 5 and P5. Since the lone female founder #5 was pregnant when captured, a founder—here designated P5—was assigned to the studbook as her mate. This pedigree was drawn using the pedigree analysis software package Pedpack, version 2.0.

Once the pedigree was constructed, genetic analysis of the population could begin. These analyses were conducted using the SPARKS population management software package. Inbreeding coefficients and mean kinships¹ were calculated for each animal, with ordered lists of animals sorted by their mean kinships (Table 1). The average inbreeding coefficient in this population is 0.106, while the average population mean kinship is 0.208. This level of inbreeding is slightly less than that expected in a mating between half-siblings. On the other hand, this level of inbreeding is considerably less than that seen in many other captive populations of mammals.

The proportional genetic contribution made by each founder to the current population was also determined (Table 2). Through this analysis, it is clear that the lone founding female #5 (as expected) contributes substantially to the current population, with founder #P5 contributing significantly less. Gene-drop analysis of the pedigree (Table 3) reveals that founder #5 is markedly over-represented relative to the ideal, or "target" level of genetic representation, while founders #2 and #P5 are under-represented. The analysis also shows that, in part because of this deviation from ideal founder representation, 79% of the original wild gene diversity or heterozygosity contained in the founder group has been retained in the current population.

¹For definitions of these and other terms throughout this report, please refer to the original Heritage Fund Grant Application submitted by the author.

Finally, founder gene extinction analysis using Pedpack was done to assess the number of unique founder alleles remaining in the current population. The results of this analysis are shown in Table 4. Overall, if we start with 8 unique alleles at a given genetic locus in the pedigree (2 per founder), the analysis reveals that 7.03 of these alleles (averaged across all loci) remain in the current population. As Table 4 shows, 99.95% of founder #2's alleles have survived, while only 61.9% have survived from founder #P5. The distribution of gene survival density indicates that, at almost 60% of the loci in the current descendent population, both alleles from founders #2, #5, and #11 have survived, while one allele from founder #P5 has been lost.

A discussion has existed for many years concerning the true number of founders in the Mexican wolf captive population. It has been proposed that presumed founder #2 was in fact a son of founder #5, thereby reducing the actual number of founding wolves from four to three. Research is currently being conducted by molecular geneticists in an attempt to finally resolve this issue. In the meantime, however, I decided to repeat the analyses described above on a modified pedigree in which #2 was a full sibling to littermates #6-10, i.e., was an offspring of founders #5 and #P5 (full results not shown). The mean inbreeding coefficient rose substantially, to 0.184; furthermore, the over-representation of founder #5 increased, leading to a slightly greater loss of founder gene diversity in the descendent population. Gene extinction analysis revealed a total of 5.42 out of 6 unique founder alleles surviving in the current population under the three-founder scenario.

Much of these data are summarized in a slightly different fashion in Figure 2, which shows the mean inbreeding coefficient, proportional retention of gene diversity (GD), and the number of surviving founder alleles through time, starting with the founding of the captive population around 1977. Under both founder number scenarios, we see a rise in the mean inbreeding coefficient beginning in 1983 and continuing until today. The fairly dramatic increase in founder allele survival seen in 1980 results from the inclusion of founder #11, thereby immediately adding two new founder alleles to the population. Likewise, the noticeable reduction in gene survival seen in 1992 results from the death of founder #2 at the age of fifteen.

II. Inbreeding Depression in the Captive Population

A major component of the study is to assess the level of inbreeding depression in the captive Mexican wolf population. This is done by evaluating a specific fitness trait among both inbred and non-inbred wolves. Given the nature of the available studbook data, I have chosen to initially focus on longevity as the appropriate fitness character. I am currently in the process of accumulating data on age and cause of death for each deceased wolf. If such data are not readily available in the studbook records, I will contact the specific institution housing the animals when they died to determine specific cause of death. Once these data are collected, standard regression analyses will be employed to determine if inbreeding depression exists for this character.

In addition to death data, a number of wolves were weighed at specific ages during routine medical exams. It is possible to use these data as well to determine if inbreeding depression exists in the form of reduced body size among the members of the captive population. Accumulation of these data is likewise proceeding.

Unfortunately, litter size data, potentially valuable for such inbreeding studies, do not exist for this population. It is therefore necessary to use the aforementioned longevity and weight data in the inbreeding depression analysis.

III. Microsatellite Data and Pedigree Analysis

Dr. Robert Wayne of UCLA has conducted a survey of variability at a number of microsatellite loci in the captive Mexican wolf population. Microsatellite loci consist of a variable number of repeats of short nucleotide sequences and evolve through the gain or loss of repeat units, rather than direct nucleotide substitutions. Because of their relatively high mutation rates, microsatellite loci can be used to detect differentiation among small populations that cannot be revealed by analysis of more slowly evolving loci. Dr. Wayne has used this technique to assess the genetic purity of both the certified wolf lineage as well as the two uncertified lineages.

Dr. Phil Hedrick of ASU and I have initiated a collaboration with Dr. Wayne in which we will use his microsatellite data in combination with pedigree analysis methodology to assess the genetic composition of specific loci in the founding wolves. More specifically, we will be able to assign founder allele frequencies to specific microsatellite loci, and trace the transmission of these alleles through the wolf pedigree. This type of sophisticated analysis will provide valuable insight into the nature and constitution of molecular genetic variation in small populations. Efforts are currently underway to construct a database of genotypes for the 10 microsatellite loci Wayne has studied. Once completed, this database will be incorporated into the pedigree analysis package Pedpack to identify likely founder genotypes for each of the loci.

Figure 1. Marriage node pedigree for the captive Mexican wolf population. Data are current through 7 July, 1994. Circles designate females; squares designate males. Black symbols indicate living animals. Numbers above each symbol are the studbook ID numbers for each animal.

Mexican Wolf: Data through 7 July 1994

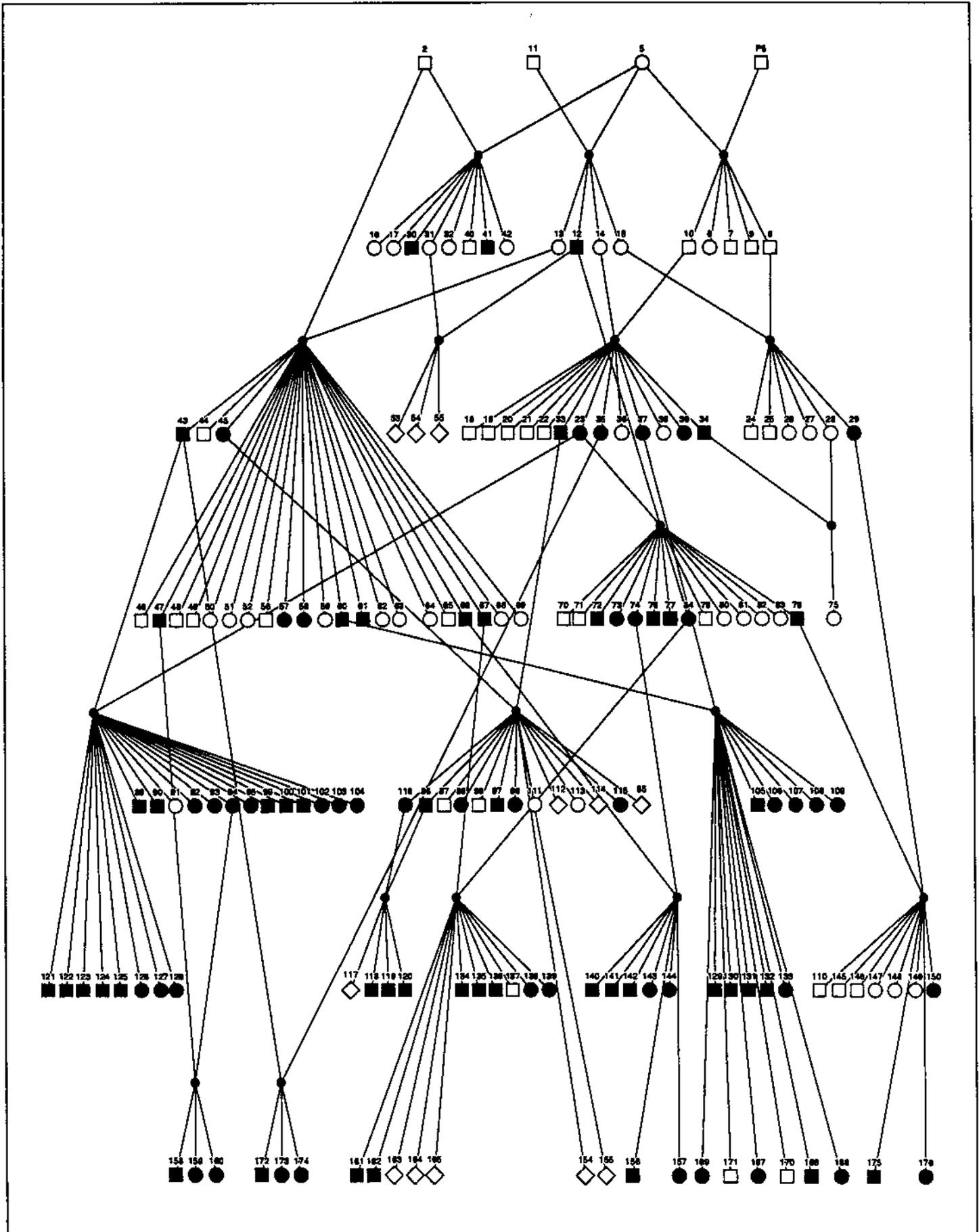


Figure 2. The mean inbreeding coefficient (F), proportional founder gene diversity (GD) retained, and the number of surviving founder genes in the captive Mexican wolf population from its beginnings in 1977 through 1993. Both founder number scenarios are included in the graphs.

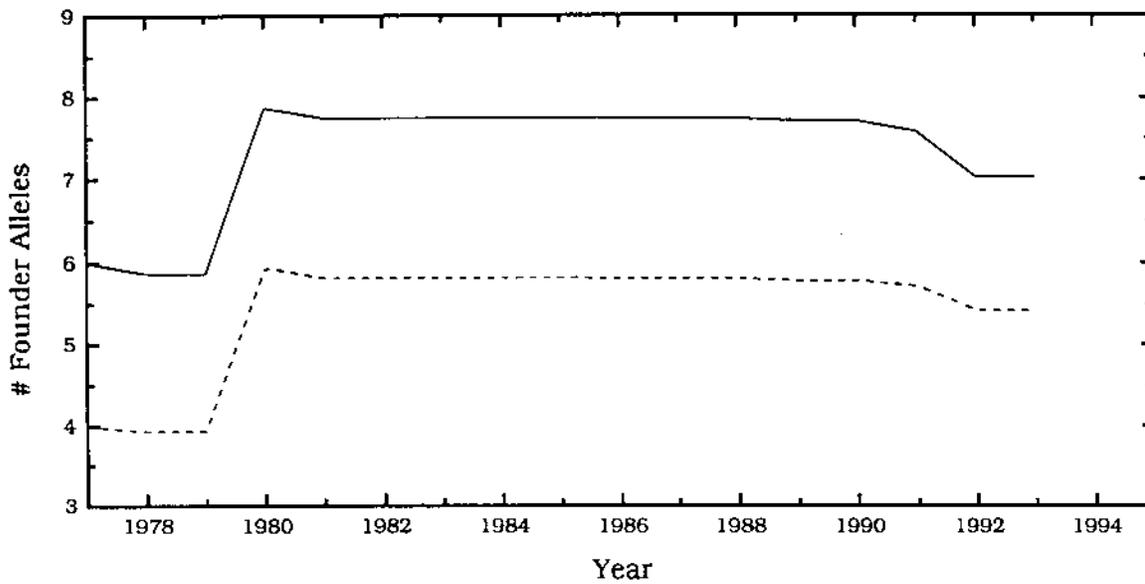
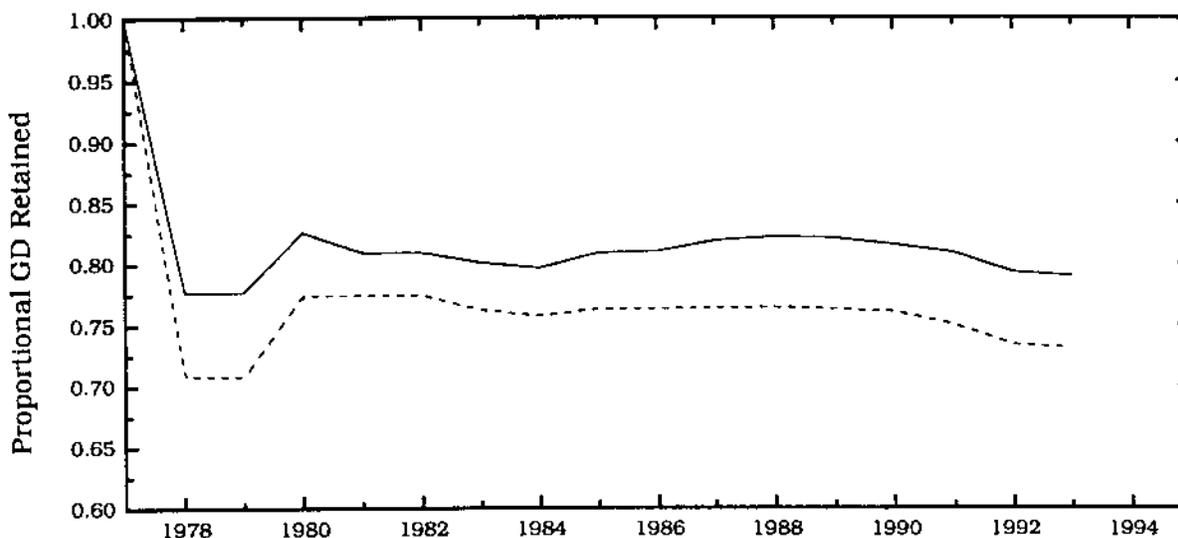
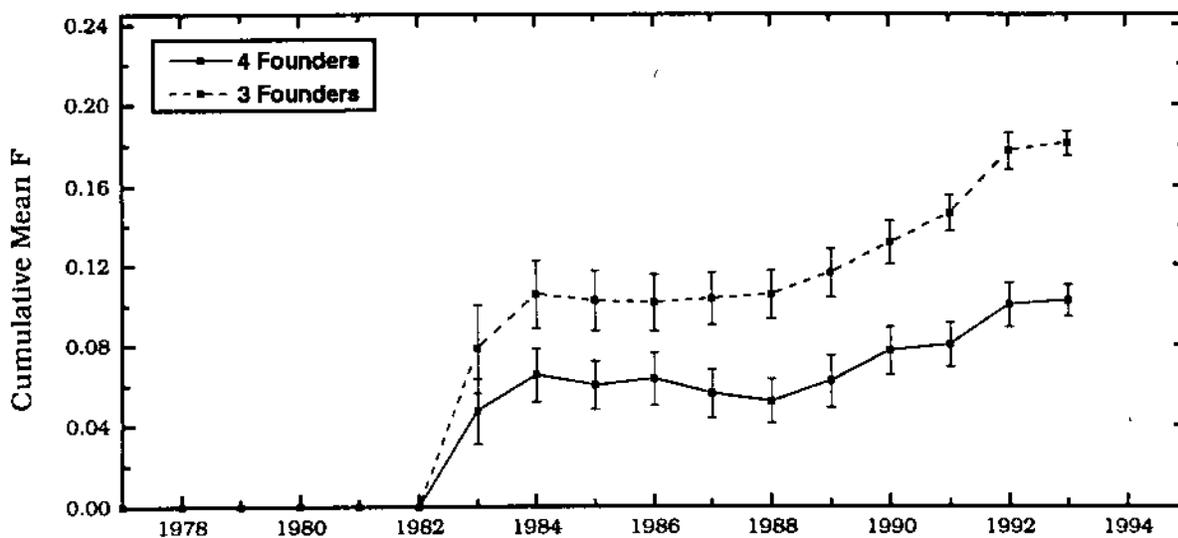


Table 1. Inbreeding coefficients and mean kinships for the Mexican wolf captive population as of 7 July, 1994.

Inbreeding coefficients and mean kinships for
mexwolf Thu Jul 21 21:14:33 1994

Inbreeding and kinship calculations assume UNKNOWNs are founders.

MEAN KINSHIP OF LIVING ANIMALS TO LIVING NON-FOUNDERS

STUDBOOK	SIRE	DAM	INBREEDING	MEAN KINSHIP	GENOME KNOWN
12 M	11	5	F = 0.0000	mk = 0.1878	1.0000
23 F	10	14	F = 0.1250	mk = 0.2481	1.0000
29 F	8	15	F = 0.1250	mk = 0.1543	1.0000
30 M	2	5	F = 0.0000	mk = 0.1585	1.0000
33 M	10	14	F = 0.1250	mk = 0.2100	1.0000
34 M	10	14	F = 0.1250	mk = 0.1978	1.0000
35 F	10	14	F = 0.1250	mk = 0.2019	1.0000
37 F	10	14	F = 0.1250	mk = 0.2168	1.0000
39 F	10	14	F = 0.1250	mk = 0.1978	1.0000
41 M	2	5	F = 0.0000	mk = 0.1585	1.0000
43 M	2	13	F = 0.0000	mk = 0.2048	1.0000
45 F	2	13	F = 0.0000	mk = 0.1838	1.0000
47 M	2	13	F = 0.0000	mk = 0.1756	1.0000
57 F	2	13	F = 0.0000	mk = 0.1716	1.0000
58 F	2	13	F = 0.0000	mk = 0.1716	1.0000
60 M	2	13	F = 0.0000	mk = 0.1906	1.0000
61 M	2	13	F = 0.0000	mk = 0.1716	1.0000
66 M	2	13	F = 0.0000	mk = 0.1811	1.0000
67 M	2	13	F = 0.0000	mk = 0.1811	1.0000
72 M	12	23	F = 0.1875	mk = 0.2205	1.0000
73 F	12	23	F = 0.1875	mk = 0.2205	1.0000
74 F	12	23	F = 0.1875	mk = 0.2294	1.0000
76 M	12	23	F = 0.1875	mk = 0.2205	1.0000
77 M	12	23	F = 0.1875	mk = 0.2205	1.0000
78 M	12	23	F = 0.1875	mk = 0.2243	1.0000
84 F	12	23	F = 0.1875	mk = 0.2294	1.0000
86 M	33	45	F = 0.0938	mk = 0.2033	1.0000
88 F	33	45	F = 0.0938	mk = 0.1995	1.0000
89 M	43	23	F = 0.0938	mk = 0.2290	1.0000
90 M	43	23	F = 0.0938	mk = 0.2290	1.0000
92 F	43	23	F = 0.0938	mk = 0.2290	1.0000
93 F	43	23	F = 0.0938	mk = 0.2290	1.0000
94 F	43	23	F = 0.0938	mk = 0.2328	1.0000
95 F	43	23	F = 0.0938	mk = 0.2290	1.0000
97 M	33	45	F = 0.0938	mk = 0.1995	1.0000
98 F	33	45	F = 0.0938	mk = 0.1995	1.0000
99 M	43	23	F = 0.0938	mk = 0.2290	1.0000
100 M	43	23	F = 0.0938	mk = 0.2290	1.0000
101 M	43	23	F = 0.0938	mk = 0.2290	1.0000
102 F	43	23	F = 0.0938	mk = 0.2290	1.0000
103 F	43	23	F = 0.0938	mk = 0.2290	1.0000
104 F	43	23	F = 0.0938	mk = 0.2290	1.0000
105 M	60	37	F = 0.0938	mk = 0.2063	1.0000
106 F	60	37	F = 0.0938	mk = 0.2063	1.0000
107 F	60	37	F = 0.0938	mk = 0.2063	1.0000
108 F	60	37	F = 0.0938	mk = 0.2063	1.0000

Table 1 (Cont'd.)

STUDBOOK	SIRE	DAM	INBREEDING	MEAN KINSHIP	GENOME KNOWN
109 F	60	37	F = 0.0938	mk = 0.2063	1.0000
115 F	33	45	F = 0.0938	mk = 0.1995	1.0000
116 F	33	45	F = 0.0938	mk = 0.2033	1.0000
118 M	86	116	F = 0.3126	mk = 0.2058	1.0000
119 M	86	116	F = 0.3126	mk = 0.2058	1.0000
120 M	86	116	F = 0.3126	mk = 0.2058	1.0000
121 M	43	23	F = 0.0938	mk = 0.2290	1.0000
122 M	43	23	F = 0.0938	mk = 0.2290	1.0000
123 M	43	23	F = 0.0938	mk = 0.2290	1.0000
124 M	43	23	F = 0.0938	mk = 0.2290	1.0000
125 M	43	23	F = 0.0938	mk = 0.2290	1.0000
126 F	43	23	F = 0.0938	mk = 0.2290	1.0000
127 F	43	23	F = 0.0938	mk = 0.2290	1.0000
128 F	43	23	F = 0.0938	mk = 0.2290	1.0000
129 M	60	37	F = 0.0938	mk = 0.2063	1.0000
130 M	60	37	F = 0.0938	mk = 0.2063	1.0000
131 M	60	37	F = 0.0938	mk = 0.2063	1.0000
132 M	60	37	F = 0.0938	mk = 0.2063	1.0000
133 F	60	37	F = 0.0938	mk = 0.2063	1.0000
134 M	67	84	F = 0.1094	mk = 0.2077	1.0000
135 M	67	84	F = 0.1094	mk = 0.2077	1.0000
136 M	67	84	F = 0.1094	mk = 0.2077	1.0000
138 F	67	84	F = 0.1094	mk = 0.2077	1.0000
139 F	67	84	F = 0.1094	mk = 0.2077	1.0000
140 M	66	74	F = 0.1094	mk = 0.2077	1.0000
141 M	66	74	F = 0.1094	mk = 0.2077	1.0000
142 M	66	74	F = 0.1094	mk = 0.2077	1.0000
143 F	66	74	F = 0.1094	mk = 0.2077	1.0000
144 F	66	74	F = 0.1094	mk = 0.2077	1.0000
150 F	78	29	F = 0.1875	mk = 0.1916	1.0000
156 M	66	74	F = 0.1094	mk = 0.2077	1.0000
157 F	66	74	F = 0.1094	mk = 0.2077	1.0000
158 M	47	94	F = 0.1719	mk = 0.2068	1.0000
159 F	47	94	F = 0.1719	mk = 0.2068	1.0000
160 F	47	94	F = 0.1719	mk = 0.2068	1.0000
161 M	67	84	F = 0.1094	mk = 0.2077	1.0000
162 M	67	84	F = 0.1094	mk = 0.2077	1.0000
166 M	60	37	F = 0.0938	mk = 0.2063	1.0000
167 F	60	37	F = 0.0938	mk = 0.2063	1.0000
168 F	60	37	F = 0.0938	mk = 0.2063	1.0000
169 F	60	37	F = 0.0938	mk = 0.2063	1.0000
172 M	43	35	F = 0.0938	mk = 0.2059	1.0000
173 F	43	35	F = 0.0938	mk = 0.2059	1.0000
174 F	43	35	F = 0.0938	mk = 0.2059	1.0000
175 M	78	29	F = 0.1875	mk = 0.1916	1.0000
176 F	78	29	F = 0.1875	mk = 0.1916	1.0000

Table 1 (Cont'd.)

ORDERED LISTS OF MEAN KINSHIP BY SEX:

Rank	MALES	MK	Age	Known	FEMALES	MK	Age	Known
1	30	0.1585	11	1.000	29	0.1543	11	1.000
2	41	0.1585	10	1.000	57	0.1716	7	1.000
3	61	0.1716	6	1.000	58	0.1716	7	1.000
4	47	0.1756	8	1.000	45	0.1838	9	1.000
5	66	0.1811	5	1.000	176	0.1916	0	1.000
6	67	0.1811	5	1.000	150	0.1916	1	1.000
7	12	0.1878	13	1.000	39	0.1978	10	1.000
8	60	0.1906	6	1.000	88	0.1995	4	1.000
9	175	0.1916	0	1.000	98	0.1995	3	1.000
10	34	0.1978	10	1.000	115	0.1995	2	1.000
11	97	0.1995	3	1.000	35	0.2019	10	1.000
12	86	0.2033	4	1.000	116	0.2033	6	1.000
13	43	0.2048	9	1.000	173	0.2059	0	1.000
14	118	0.2058	2	1.000	174	0.2059	0	1.000
15	119	0.2058	2	1.000	167	0.2063	0	1.000
16	120	0.2058	2	1.000	168	0.2063	0	1.000
17	172	0.2059	0	1.000	169	0.2063	0	1.000
18	166	0.2063	0	1.000	106	0.2063	2	1.000
19	105	0.2063	2	1.000	107	0.2063	2	1.000
20	129	0.2063	1	1.000	108	0.2063	2	1.000
21	130	0.2063	1	1.000	109	0.2063	2	1.000
22	131	0.2063	1	1.000	133	0.2063	1	1.000
23	132	0.2063	1	1.000	159	0.2068	0	1.000
24	158	0.2068	0	1.000	160	0.2068	0	1.000
25	140	0.2077	1	1.000	143	0.2077	1	1.000
26	141	0.2077	1	1.000	144	0.2077	1	1.000
27	142	0.2077	1	1.000	157	0.2077	0	1.000
28	156	0.2077	0	1.000	138	0.2077	1	1.000
29	134	0.2077	1	1.000	139	0.2077	1	1.000
30	135	0.2077	1	1.000	37	0.2168	10	1.000
31	136	0.2077	1	1.000	73	0.2205	5	1.000
32	161	0.2077	0	1.000	92	0.2290	3	1.000
33	162	0.2077	0	1.000	93	0.2290	3	1.000
34	33	0.2100	10	1.000	95	0.2290	3	1.000
35	72	0.2205	5	1.000	102	0.2290	2	1.000
36	76	0.2205	4	1.000	103	0.2290	2	1.000
37	77	0.2205	4	1.000	104	0.2290	2	1.000
38	78	0.2243	4	1.000	126	0.2290	1	1.000
39	89	0.2290	3	1.000	127	0.2290	1	1.000
40	90	0.2290	3	1.000	128	0.2290	1	1.000
41	99	0.2290	2	1.000	84	0.2294	4	1.000
42	101	0.2290	2	1.000	74	0.2294	5	1.000
43	100	0.2290	2	1.000	94	0.2328	3	1.000
44	121	0.2290	1	1.000	23	0.2481	11	1.000
45	122	0.2290	1	1.000				
46	123	0.2290	1	1.000				
47	124	0.2290	1	1.000				
48	125	0.2290	1	1.000				

GENETIC SUMMARY OF POPULATION

Descendant population Mean Kinship: 0.2081
 Gene Diversity: 0.7919
 Founder Genome Equivalents: 2.4030

Table 2. Founder bloodline composition for each living Mexican wolf. Summary statistics at the end indicate the proportional contribution of each founder to the living population.

FOUNDER analysis for
mexwolf Thu Jul 21 21:14:33 1994

Founder representation in each living animal:
 Founders listed across top, descendants down side.
 Founder studbook numbers in parentheses indicate UNKNOWNs.
 Studbook numbers beginning with P indicate wild or unknown founders that
 mated with studbook # without the P to produce CB offspring.

Founders	2	5	11	P5
12	0.0000	0.5000	0.5000	0.0000
23	0.0000	0.5000	0.2500	0.2500
29	0.0000	0.5000	0.2500	0.2500
30	0.5000	0.5000	0.0000	0.0000
33	0.0000	0.5000	0.2500	0.2500
34	0.0000	0.5000	0.2500	0.2500
35	0.0000	0.5000	0.2500	0.2500
37	0.0000	0.5000	0.2500	0.2500
39	0.0000	0.5000	0.2500	0.2500
41	0.5000	0.5000	0.0000	0.0000
43	0.5000	0.2500	0.2500	0.0000
45	0.5000	0.2500	0.2500	0.0000
47	0.5000	0.2500	0.2500	0.0000
57	0.5000	0.2500	0.2500	0.0000
58	0.5000	0.2500	0.2500	0.0000
60	0.5000	0.2500	0.2500	0.0000
61	0.5000	0.2500	0.2500	0.0000
66	0.5000	0.2500	0.2500	0.0000
67	0.5000	0.2500	0.2500	0.0000
72	0.0000	0.5000	0.3750	0.1250
73	0.0000	0.5000	0.3750	0.1250
74	0.0000	0.5000	0.3750	0.1250
76	0.0000	0.5000	0.3750	0.1250
77	0.0000	0.5000	0.3750	0.1250
78	0.0000	0.5000	0.3750	0.1250
84	0.0000	0.5000	0.3750	0.1250
86	0.2500	0.3750	0.2500	0.1250
88	0.2500	0.3750	0.2500	0.1250
89	0.2500	0.3750	0.2500	0.1250
90	0.2500	0.3750	0.2500	0.1250
92	0.2500	0.3750	0.2500	0.1250
93	0.2500	0.3750	0.2500	0.1250
94	0.2500	0.3750	0.2500	0.1250
95	0.2500	0.3750	0.2500	0.1250
97	0.2500	0.3750	0.2500	0.1250
98	0.2500	0.3750	0.2500	0.1250
99	0.2500	0.3750	0.2500	0.1250
100	0.2500	0.3750	0.2500	0.1250
101	0.2500	0.3750	0.2500	0.1250
102	0.2500	0.3750	0.2500	0.1250
103	0.2500	0.3750	0.2500	0.1250
104	0.2500	0.3750	0.2500	0.1250
105	0.2500	0.3750	0.2500	0.1250
106	0.2500	0.3750	0.2500	0.1250
107	0.2500	0.3750	0.2500	0.1250

Table 2 (Cont'd.)

Founders	2	5	11	P5
108	0.2500	0.3750	0.2500	0.1250
109	0.2500	0.3750	0.2500	0.1250
115	0.2500	0.3750	0.2500	0.1250
116	0.2500	0.3750	0.2500	0.1250
118	0.2500	0.3750	0.2500	0.1250
119	0.2500	0.3750	0.2500	0.1250
120	0.2500	0.3750	0.2500	0.1250
121	0.2500	0.3750	0.2500	0.1250
122	0.2500	0.3750	0.2500	0.1250
123	0.2500	0.3750	0.2500	0.1250
124	0.2500	0.3750	0.2500	0.1250
125	0.2500	0.3750	0.2500	0.1250
126	0.2500	0.3750	0.2500	0.1250
127	0.2500	0.3750	0.2500	0.1250
128	0.2500	0.3750	0.2500	0.1250
129	0.2500	0.3750	0.2500	0.1250
130	0.2500	0.3750	0.2500	0.1250
131	0.2500	0.3750	0.2500	0.1250
132	0.2500	0.3750	0.2500	0.1250
133	0.2500	0.3750	0.2500	0.1250
134	0.2500	0.3750	0.3125	0.0625
135	0.2500	0.3750	0.3125	0.0625
136	0.2500	0.3750	0.3125	0.0625
138	0.2500	0.3750	0.3125	0.0625
139	0.2500	0.3750	0.3125	0.0625
140	0.2500	0.3750	0.3125	0.0625
141	0.2500	0.3750	0.3125	0.0625
142	0.2500	0.3750	0.3125	0.0625
143	0.2500	0.3750	0.3125	0.0625
144	0.2500	0.3750	0.3125	0.0625
150	0.0000	0.5000	0.3125	0.1875
156	0.2500	0.3750	0.3125	0.0625
157	0.2500	0.3750	0.3125	0.0625
158	0.3750	0.3125	0.2500	0.0625
159	0.3750	0.3125	0.2500	0.0625
160	0.3750	0.3125	0.2500	0.0625
161	0.2500	0.3750	0.3125	0.0625
162	0.2500	0.3750	0.3125	0.0625
166	0.2500	0.3750	0.2500	0.1250
167	0.2500	0.3750	0.2500	0.1250
168	0.2500	0.3750	0.2500	0.1250
169	0.2500	0.3750	0.2500	0.1250
172	0.2500	0.3750	0.2500	0.1250
173	0.2500	0.3750	0.2500	0.1250
174	0.2500	0.3750	0.2500	0.1250
175	0.0000	0.5000	0.3125	0.1875
176	0.0000	0.5000	0.3125	0.1875
Founders	2	5	11	P5
Founder contributions	21.6250	35.6875	24.6875	10.0000
Fractional contributions	0.2351	0.3879	0.2683	0.1087

Table 3. Gene drop analysis for the captive Mexican wolf population. See text for explanation.

GENE DROP analysis for
mexwolf Thu Jul 21 21:14:33 1994

FOUNDER ALLELE REPRESENTATION

Founder	Retention	%Representation	Target	Difference
2 M	1.000	23.496	28.419	4.922
5 F	0.992	38.772	28.191	-10.581
11 M	0.905	26.853	25.722	-1.130
P5 M	0.621	10.879	17.668	6.789

GENETIC SUMMARY

LIVING DESCENDANT POPULATION

POTENTIAL

Number of founders:	4	4
Mean retention:	0.879	0.879
Founder genomes surviving:	3.517	3.517
Founder Genome Equivalents:	2.410	3.517
Fraction of wild gene diversity retained:	0.792	0.858
Fraction of wild gene diversity lost:	0.208	0.142
Mean inbreeding coefficient:	0.106	

Table 4. Founder gene survival probabilities in the captive Mexican wolf population. The gene survival density gives the joint probability distribution of the number of surviving genes from each founder. The majority of the density (59%) is contained in the situation where only one gene is lost from founder #P5.

GENE SURVIVAL, MEXICAN WOLF
Data through 7 July, 1994

Founder	Means
#2	1.999
#5	1.981
#11	1.809
#P5	1.238
Total	7.027

2	5	11	P5	Density
1	1	1	0	0.0000000154
2	1	1	0	0.0000157817
1	2	1	0	0.0000014403
2	2	1	0	0.0014734003
1	1	2	0	0.0000000756
2	1	2	0	0.0000772922
1	2	2	0	0.0000060981
2	2	2	0	0.0062383964
1	1	1	1	0.0000020055
2	1	1	1	0.0020516167
1	2	1	1	0.0001370139
2	2	1	1	0.1401652604
1	1	2	1	0.0000098221
2	1	2	1	0.0100479868
1	2	2	1	0.0005797657
2	2	2	1	0.5931002790
1	1	1	2	0.0000009873
2	1	1	2	0.0010100267
1	2	1	2	0.0000448673
2	2	1	2	0.0458992050
1	1	2	2	0.0000048355
2	1	2	2	0.0049467012
1	2	2	2	0.0001896359
2	2	2	2	0.1939974912