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Canine Parvovirus Enteritis, Canine Distemper, and Major Histocompatibility Complex Genetic Variation in Mexican Wolves

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ABSTRACT: The endangered Mexican wolf (*Canis lupus baileyi*) was recently reintroduced into Arizona and New Mexico (USA). In 1999 and 2000, pups from three litters that were part of the reintroduction program died of either canine parvovirus or canine distemper. Overall, half (seven of 14) of the pups died of either canine parvovirus or canine distemper. The parents and their litters were analyzed for variation at the class II major histocompatibility complex (MHC) gene *DRB*1. Similar MHC genes are related to disease resistance in other species. All six of the surviving pups genotyped for the MHC gene were heterozygous while five of the pups that died were heterozygous and one was homozygous. Resistance to pathogens is an important aspect of the management and long-term survival of endangered taxa, such as the Mexican wolf.

Key words: Canine distemper, canine parvovirus, *Canis lupus baileyi,* endangered species, genetics, inbreeding, wolves.

In recent years, it has become widely recognized that endangered species may be threatened by exposure to pathogens (Lyles and Dobson, 1993; Laurenson et al., 1998; Murray et al., 1999; Woodroffe, 1999; Lafferty and Gerber, 2002), some of them novel to the species. A pathogen introduced from a reservoir in a more common species, such as domestic animals, may result in final decline to extinction, particularly if the endangered species has low disease resistance. Understanding the cause of such low disease resistance, whether it results from lack of previous exposure, low genetic resistance, environmental stress, or other factors, is important for management decisions to avoid extinction of rare species.

Measuring differential levels of resistance in endangered species is difficult because experimental exposure is generally not possible (however, see Hedrick et al., 2001; Arkush et al., 2002). In wild populations of endangered species, generally it is even more difficult to determine differential resistance to infectious disease because the primary cause of mortality is often not known. Furthermore, samples sizes of the number of individuals surviving or dying from exposure to disease with different attributes, such as genetic variants, are generally small.

The major histocompatibility complex (MHC) is one of the most important genetic systems for infectious disease resistance in vertebrates (Edwards and Hedrick, 1998; Hedrick and Kim, 2000; Hill, 2001). More specifically, MHC molecules are an essential part of the adaptive immune system. Major histocompatibility complex molecules bind small peptide fragments, which may be derived from pathogens, and this MHC-peptide complex may result in the initiation of an immune response that specifically reacts to the foreign bound peptide (for details, see Janeway et al., 1999).

Association of disease resistance and MHC variation has been difficult to document (Hedrick and Kim, 2000), but a number of studies in humans have documented its importance for resistance to malaria (Hill et al., 1991), hepatitis (Thurz et al., 1997), and acquired immunodeficiency syndrome (Carrington et al., 1999). In addition, O'Brien and Evermann (1988) suggested that organisms with low MHC variation, such as many endangered species, might have high susceptibility to infectious disease (see also Edwards and Potts, 1996).

The Mexican wolf (*Canis lupus baileyi*) is a subspecies of the gray wolf based on molecular genetic examination (Garcia-Moreno et al., 1996; Hedrick et al., 1997).

TABLE 1. Three litters of Mexican wolves (a, b, and c) exposed to either canine parvovirus or canine distemper virus and their genotypes at class II MHC gene *DRB*1.

Litter, pathogen exposure (birthdate)	Parents[a]		Offspring[a]	
	Female	Male	Survived	Died
a, parvovirus (May 1999)	11[b] (F191)	22[b] (M208)	12[b] (F624)	12 (M623)
			12 (M627)	12 (F625)
			12 (F628)	12 (M626)
b, parvovirus (1 May 1999)	11 (F511)	12 (M509)	12 (F587)	12 (F588)
			12 (M590)	—[c](M589)
c, distemper (27 April 1999)	12 (F168)	11 (M183)	—[c](M583)	12 (M582)
			12 (M584)	11 (F585)

[a] Sex and studbook numbers are in parentheses.
[b] Alleles *Calu*-1 and *Calu*2- from Hedrick et al. (2000) are indicated by 1 and 2.
[c] — indicates that it was not possible to determine the MHC genotype.

There have been no confirmed sightings of wild Mexican wolves for over two decades. The only extant Mexican wolves are the approximately 240 in captivity and the reintroduced population that was established in recent years in southeast Arizona and subsequently southwest New Mexico (USA) from this captive population. Originally all the reintroduced animals were from the McBride lineage, previously known as the Certified lineage, but in recent years animals with ancestry from the Aragon and Ghost Ranch lineages have been released (see Hedrick et al., 1997 for information on the different Mexican wolf lineages). Hedrick et al. (2000) examined the extent of genetic variation for the class II MHC gene *DRB*1 in the McBride lineage and found two alleles, *Calu*-1 and *Calu*-2, which had frequencies of 0.35 and 0.65, respectively. These two alleles were quite divergent and differed by 10 amino acids of 69 with most of these differences at the functionally important antigen binding site, suggesting that in the past they have been under strong balancing selection, presumably related to pathogen resistance.

In 1999, mortalities occurred in two litters of Mexican wolves from canine parvovirus enteritis (a and b) and in another litter (c) from canine distemper (Table 1). Litter a, wild born in May 1999 in Greenlee County near Four Bar Mesa, Arizona (USA) was part of the Pipestem pack. Because of livestock depredation, traps were set in July 1999 to capture and move this pack. Two of the pups, M623 and F624 (M or F indicates male or female and the number indicates the studbook number), were caught on 23 July and 11 August, respectively, and were housed together in a pen. On 24 August, pup M623 was found dead. Carcasses of all pups were sent to the National Wildlife Health Center (NWHC; Madison, Wisconsin, USA) for necropsy. Later that day, three other pups (F625, M626, and M627) from the litter were captured and all surviving pups were taken to the United States Fish and Wildlife Service (USFWS) Mexican wolf facility located on the Sevilleta National Wildlife Refuge in New Mexico. Another pup, F628, and the alpha female were not captured until December 1999.

None of the pups had been vaccinated. On 27 August, a veterinarian examined them, found no indications of illness, and vaccinated the pups. However, on 30 August pups F625 and M626 were found dead in the pen at Sevilleta. Laboratory analysis indicated that the three pups had died from canine parvovirus enteritis. Although the precise source of the parvovirus infection is not known, it is suspected that contact with unvaccinated dogs or their feces in the remote area where the

reintroduced population is established may have been the source of transmission.

Because of the deaths from parvovirus infection in litter a, there was concern about another litter b at Sevilleta of four pups born on 1 May 1999. These pups had not been vaccinated because of an attempt to limit their exposure to humans as much as possible prior to release to the wild. These pups were vaccinated on 1 September in response to mortality in litter a. On 7 September, two of these pups, F588 and M589, were found dead; littermates, F587 and M590, survived. Although the exact mode of transmission of parvovirus to litter b is not known, it is likely that it was transmitted from litter a, either indirectly or by the caretaker, before the diagnosis of parvovirus was known. Canine parvovirus enteritis was subsequently diagnosed in these animals at NWHC.

Litter c, composed of five pups (M582, M583, M584, F585, and M586) was born in the Coalson acclimation pen, near the confluence of the Blue River and the San Francisco River (Arizona) on 27 April 1999. These pups, their parents, and a yearling male (M555) composed the eight members of the Gavilan Pack that was released on 21 May 1999. Because of livestock depredation, the pack was recaptured in late 1999 and early 2000 (except yearling M555 and pup M586 whose fate is unknown) and all the pups were vaccinated for the first time upon capture. Brown staining on their lower incisors, possibly indicative of an encounter with distemper at a young age, was noted on the recapture forms of M582, M584, and F585.

On 24 February 2000, F585 was acting abnormally, racing and stumbling around the pen, snapping at other wolves, and with saliva coming from her mouth. Wolf M582 was unable to move with mucoid fluid dripping from his mouth. Clinical examination demonstrated "ticking" of the right hind leg, twitching of the ears, and convulsive snapping of the jaws. Canine distemper was diagnosed and after consultation with U.S. Fish and Wildlife Service (USFWS) and M582 was euthanized.

Over the next few months, F585 appeared to recover from her abnormal behavior. However, on 15 August 2000 she was found in very poor condition, dragging her back legs, and shaking uncontrollably. She was diagnosed as having canine distemper encephalitis and was subsequently euthanized. The diagnosis of canine distemper in both of the wolves was verified by the NWHC. Overall 50% (seven of 14) of the pups died.

We extracted DNA from blood (Epicentre Technologies, Madison, Wisconsin) taken from the parents of the three litters and samples from the surviving animals. Tissue samples from the pups that died of disease were acquired from a USFWS Mexican wolf sample repository at the University of New Mexico (Albuquerque, New Mexico). Genotypes were determined using single strand confirmation polymorphism as in Hedrick et al. (2000).

We were able to obtain genotypes for the parents of the three litters (Table 1). For litters b and c, one of the parents was homozygous for *Calu*-1 and the other was heterozygous for *Calu*-1 and *Calu*-2. In other words, our expectation was that 50% of the progeny from both of these litters should be homozygotes and 50% heterozygotes, the optimal situation for examining differential genetic disease resistance. Unfortunately, in litter a the parents were homozygotes for different alleles so that all their progeny were expected to be heterozygous, i.e., there was no segregation within the litter at this MHC locus.

We were able to obtain product for six of seven surviving offspring and six of seven offspring that died. As expected, in litter a all the pups were heterozygotes. In litter b the three offspring for which we were able to obtain genotypes were heterozygotes (two survived and one died) so that no indication of differential genetic resistance could be determined. In litter c, genetic segregation was observed with two heterozygotes and one homozygote in the

three pups for which we could obtain genotypes. One heterozygote survived distemper and a heterozygote and a homozygote died.

Higher survival of heterozygotes than homozygotes has been observed in experiments with infectious hematopoeitic necrosis virus in salmon (Arkush et al., 2002) and in surveys of humans with human immunodeficiency virus (Carrington et al., 1999) or hepatitis (Thurz et al., 1997). Even though the sample size in our study was small, such investigations are valuable because situations in which detailed genetic information is available for animals exposed to significant pathogens are uncommon. Investigations of this kind might lead to insights into natural resistance to pathogens.

The litters we studied were from the McBride lineage (Hedrick et al., 1997), four to six generations removed from the three founders. Even though this population has been managed to minimize mean kinship (Ballou et al., 1995) and consequently to generally minimize inbreeding, because of the accumulated number of generations from a few founders, the inbreeding coefficients were 0.25, 0.289, and 0.25 for litters a, b, and c, respectively. Thus the inbreeding coefficients are equivalent to those of offspring of a mating between first-degree relatives, i.e., full-siblings (Hedrick, 2000). Inbred individuals may have a greater susceptibility to pathogens (Hedrick et al., 2001; Arkush et al., 2002) so it is possible that the level of inbreeding made these animals more susceptible to pathogens than outbred individuals would have been.

Two other independent lineages, the Aragon lineage and the Ghost Ranch lineage, each with two founders (Hedrick et al., 1997), are now being combined with the McBride lineage. As a result, the level of inbreeding should be greatly reduced in wolves bred in the next few generations; offspring of crosses between the lineages would have inbreeding coefficients of 0.0. In addition, the Aragon and Ghost Ranch lineages have three different alleles at this MHC locus (Hedrick et al., 2000) so variation at this gene should be greatly enhanced by adding the two lineages.

Given the relatively high level of inbreeding in these three litters, the observed high diversity and heterozygosity at the MHC locus is reassuring. For the three parents, alleles *Calu*-1 and *Calu*-2 were in equal frequencies of 0.5 and 33% of the individuals were heterozygotes. For the progeny, alleles *Calu*-1 and *Calu*-2 were in frequencies of 0.542 and 0.458 and 92% were heterozygotes. Part of this high heterozygosity results from litter a in which all six offspring were heterozygotes because of the parental genotypes, but also because all pups in litter b were heterozygotes.

Canine parvovirus enteritis and canine distemper are major causes of mortality in wild carnivores (Deem et al., 2000; Steinel et al., 2001) and can cause mortality in free-ranging wolf populations (Johnson et al., 1994; Mech and Goyal, 1995; Mech et al., 1997). Understanding more about natural resistance to these pathogens in wild canid populations is an important aspect of the management and long-term survival of wild canid populations in general and of endangered taxa, such as Mexican wolf, in particular.

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