2006 COLUMBIA BASIN PYGMY RABBIT CAPTIVE BREEDING AND GENETIC MANAGEMENT PLAN

David Hays and Kenneth I. Warheit
Wildlife Program
Washington Department of Fish and Wildlife
600 Capitol Way N.
Olympia, WA 98501-1091

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The 2006 captive breeding and genetics management plan is an update to the 2005 plan. This plan summarizes information developed by a number of people involved with captive breeding and conservation of the Columbia Basin pygmy rabbit, including Kristin Mansfield, Jan Steele, Tara Davilla, Becky Elias, Michael Illig, Lisa Shipley, Chris Warren, Rob Westra, Lisa Harrenstien, Rod Sayler, Nina Woodford, Rachel Lamson, and Cherril Bowman.

Introduction

Pygmy rabbits (*Brachylagus idahoensis*), the smallest rabbits in North America, are found in shrub-steppe habitat within the Temperate Desert Ecoregion in western North America as described by Bailey (1998). This includes the Columbia Basin of Washington and the Columbia Plateau and Great Basin of Oregon, Idaho, Montana, Wyoming, Utah, California, and Nevada of the United States. The population in Washington is confined to the Columbia Basin of central Washington, and is estimated to have been geographically isolated from other populations of the species for thousands of years (Lyman 1991, Warheit 2001, Lyman 2004).

There are few historic accounts of pygmy rabbits in the Columbia Basin. Taylor and Shaw (1929) reported the pygmy rabbit as fairly common in Adams County, while Booth (1947) and Dalquest (1948) reported them as scarce. Pre-1962 museum specimens were collected in four counties: Adams, Grant, Douglas, and Lincoln (WDFW 1995). There were no verified pygmy rabbit collections or reports between 1962 and 1979. Little was known about the distribution and status of pygmy rabbits in Washington until surveys were conducted by Washington Department of Fish and Wildlife (WDFW, formerly Department of Game) between 1987 and 1990 (Dobler and Dixon 1990).

Pygmy rabbits were known from six relatively small, isolated populations during the 1990’s in Washington. Population sizes were never known; relative numbers of animals were estimated through counts of active burrows. Number of active burrows ranged from 10 – 590 at the six sites. The pygmy rabbit was listed as a threatened species in Washington in 1990 and was reclassified to endangered status in 1993 (WDFW 1993). A state recovery plan for the pygmy rabbit was written in 1995 (WDFW 1995).

Between 1997-2001 five of the six populations disappeared (USDI 2003). Populations with the fewest active burrows generally disappeared first. Two populations dramatically declined after fire. By March 2001, rabbits remained only at Sagebrush Flats, near Ephrata, and that population suffered a sudden large decline during the winter of 2000-2001. Reason for the sudden decline is not known.

Washington Department of Fish and Wildlife, in cooperation with Peggy Bartels, a concerned citizen, conducted genetic analyses of pygmy rabbits in 2001. The results indicated that the Columbia Basin population of pygmy rabbits is genetically distinct from all other populations of
pygmy rabbits in the United States, and appears to have suffered from a reduction in genetic
diversity over the past 50 years to a relatively low level (Warheit 2001).

Pygmy rabbits of the Columbia Basin in Washington were listed under emergency provisions of
the federal Endangered Species Act in November 2001 by the U.S. Fish and Wildlife Service,
with a final rule continuing the endangered listing in March 2003 (USDI 2003). Surveys in
winter 2003 - 2004 at Sagebrush Flats failed to locate active burrows (B. Patterson, WDFW,
pers.comm.).

Natural history of the Columbia Basin pygmy rabbit

The mean adult weights of pygmy rabbits range from 375 to slightly over 500 grams (0.83 to 1.1
pounds), and lengths from 23.5 to 29.5 cm (9.3 to 11.6 in.) (Orr 1940; Janson 1946; Wilde 1978;
Gahr 1993; WDFW 1995). Their overall color is slate-gray tipped with brown, their legs, chest,
and nape are tawny cinnamon-brown; the entire edges of their ears are pale buff. Their ears are
short, rounded, and thickly furred outside. Their tails are small, uniform in color, and nearly
unnoticeable in the wild (Orr 1940; Janson 1946; WDFW 1995). The pygmy rabbit is
distinguishable from other leporids by its small size, short ears, gray color, small hind legs, and
lack of white on the tail.

Pygmy rabbits are typically found in dense stands of sagebrush (Artemisia spp.), and are highly
dependent on sagebrush to provide both food and shelter throughout the year (Orr 1940; Green
and Flinders 1980; WDFW 1995). The winter diet of pygmy rabbits may be comprised of up to
99 percent sagebrush (Wilde 1978), which is unique among leporids (White et al. 1982). Pygmy
rabbits are known to climb among the upper branches of sage brush plants to forage (Green and
Flinders 1980).

The pygmy rabbit is the only rabbit in the United States that digs its own burrows (Nelson 1909;
Green and Flinders 1980; WDFW 1995). Pygmy rabbit burrows are typically found in deep,
loose soils. However, pygmy rabbits occasionally make use of burrows abandoned by other
species, such as the yellow-bellied marmot (Marmota flaviventris) or badger (Taxidea taxus)
(Wilde 1978; Green and Flinders 1980; WDFW 1995).

Pygmy rabbits, especially juveniles, likely use their burrows as protection from predators and
inclement weather (Bailey 1936; Bradfield 1974). The burrows frequently have multiple
entrances, some of which are concealed at the base of larger sagebrush plants (WDFW 1995).
Pygmy rabbits evade predators by maneuvering through the dense cover of their preferred
habitats, often along established trails, or by escaping into their burrows (Bailey 1936; Severaid
1950; Bradfield 1974). Burrows are relatively simple and shallow, often no more than 2 m (6.6
ft. in length and usually less than 1 m (3.3 ft) deep with no distinct chambers (Bradfield 1974;
Green and Flinders 1980; Gahr 1993). The number of active burrows may not be directly related
to the number of individuals in a given area. Some individual pygmy rabbits appear to maintain
multiple burrows, while some individual burrows are used by multiple individuals (Gahr 1993;
WDFW 1995).

Pygmy rabbits begin breeding at age 1 and breeding may occur from February through July (L.
Shipley, Washington State University, pers.comm. 2003). In some parts of the species’ range,
females may have up to three litters per year and average six young per litter (Green 1978; Wilde
Recent information on captive pygmy rabbits indicates that females may excavate specialized “natal” burrows for their litters in the vicinity of their regular burrows (P. Swenson, Oregon Zoo, pers. comm. 2001; L. Shipley, Washington State University, pers. comm. 2001). The gestation period of captive pygmy rabbits is approximately 25 days and kits emerge from their natal burrows at roughly two weeks of age (L. Shipley, Washington State University, pers. comm. 2003).

Pygmy rabbits may be active at any time of the day or night and appear to be most active during mid-morning (Bradfield 1974; Green and Flinders 1980; Gahr 1993). Remote video observation of captive pygmy rabbits indicates that they are most active at night (L. Shipley, Washington State University, pers. comm).

**History of the captive breeding program**

In December, 2000, 4 pygmy rabbits were brought from the Lemhi Valley of Idaho and reared at the Oregon Zoo in Portland. Additional rabbits were captured from Idaho in 2001 and 2002. In 2002 Idaho rabbits were moved to captive breeding facilities at Northwest Trek Wildlife Park and Washington State University. Idaho rabbits were used to develop husbandry methods, to provide animals for experimental releases (back into Idaho), and to compare reproductive results with Columbia Basin pygmy rabbits under similar captive environments.

To prevent possible extinction of the Columbia Basin pygmy rabbits, 16 pygmy rabbits were trapped at Sagebrush Flats, Washington from May 2001 through January 2002, and transported to captive breeding facilities at the Oregon Zoo and Washington State University. The captive breeding program was initiated in spring 2002 with 18 animals from the 16 founders. Captive breeding of pygmy rabbits is a cooperative project between Washington Department of Fish and Wildlife, U. S. Fish and Wildlife Service, the Oregon Zoo, Washington State University, and Northwest Trek Wildlife Park. A Science Team, with members from wildlife agencies, universities, and zoos was formed to review and direct all aspects of captive breeding.

Population growth of captive Columbia Basin pygmy rabbits has been static to negative, and only three captive Columbia Basin rabbits remain in April 2006, down from 15 in April 2005. Genetic diversity of the Columbia Basin pygmy rabbits is low, and reproductive performance has been poor compared with captive Idaho pygmy rabbits. Experimental intercrosses were conducted in 2003 to determine whether Columbia Basin and Idaho pygmy rabbits could interbreed. Results indicated that they could interbreed, and reproductive performance of intercross animals was superior to the pure Columbia Basin stock. Recovery of the Columbia Basin pygmy rabbits will require the introduction of non-Columbia Basin pygmy rabbits into the captive breeding program to improve genetic diversity and reproductive success (USFWS 2004). This approach was used for the Florida panther recovery program (Hedrick 1995).

This document summarizes results of captive breeding activities in 2002 - 2005, and outlines goals and recommendations for the endangered Columbia Basin pygmy rabbit in the captive breeding facilities in Portland, Oregon (Oregon Zoo), Pullman, Washington (Washington State University) and Eatonville, Washington (Northwest Trek Wildlife Park). We review the current genetic status of the captive population, provide the genetic goals of the captive-breeding program, and provide prescriptive recommendations that should guide the husbandry of Columbia Basin pygmy rabbits. This document has been developed to support the Columbia
Basin pygmy rabbit recovery team in their development of a recovery plan, and fulfills the requirements of Federal Recovery Permit TE050644-3.

**Captive Breeding Summary for 2001 through 2005**

Captive breeding of Idaho pygmy rabbits occurred during 2001 - 2005, while Columbia Basin pygmy rabbits were captively bred in 2002 - 2005 and intercross breeding began in 2003-, at first (2003) on an experimental basis. Captive breeding results continued to show strong differences between breeding success of Idaho pygmy rabbits and Columbia Basin pygmy rabbits. All of the breeding parameters investigated showed statistically significant differences between Idaho and Columbia Basin pygmy rabbits (Table 1). For all years, pregnancy success was 100% for Idaho females (when paired with Idaho males N = 20) and 51% for Columbia Basin females (when paired with Columbia Basin males N = 35). Pregnancy rates when Idaho or Columbia Basin females were mated to produce intercross offspring were intermediate, approximately 70% (N = 36). Perhaps most importantly, Idaho pygmy rabbits produced 1.75 litters/breeding female and 3 litters per breeding male, while Columbia Basin pygmy rabbits produced 0.66 litters per breeding female and 0.79 litters per breeding males (Table 1). Number of young produced per total number of females was 4.9 for Idaho rabbits and 2.1 for Columbia Basin pygmy rabbits (Table 1; see Elias 2004).

Fourteen 100% Columbia Basin pygmy rabbit kits lived to burrow emergence in 2002, 15 in 2003, 12 in 2004, but no 100% kits lived to burrow emergence in 2005. Extensive pairings of 100% Columbia Basin pygmy rabbits at all facilities were all eventually unsuccessful in 2005.

**Table 1. Reproductive performance of captive Idaho (2001-2005) and Columbia Basin (2002-2005) pygmy rabbits.**

<table>
<thead>
<tr>
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<th>Columbia Basin</th>
<th>Idaho</th>
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<tbody>
<tr>
<td><strong>Females pregnant</strong></td>
<td>51% (N = 35)</td>
<td>100%  (N = 20)</td>
</tr>
<tr>
<td><strong>Kits/Male</strong></td>
<td>2.38 (N = 29)</td>
<td>8.75  (N = 12)</td>
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<tr>
<td><strong>Young/Female</strong></td>
<td>2.03 (N = 34)</td>
<td>5.35  (N = 20)</td>
</tr>
<tr>
<td><strong>Pairings resulting in confirmed pregnancy</strong></td>
<td>18 % (N = 121)</td>
<td>34 % (N = 34)</td>
</tr>
<tr>
<td><strong>Litters/Breeding Female</strong></td>
<td>0.66 (N = 35)</td>
<td>1.75  (N = 20)</td>
</tr>
<tr>
<td><strong>Litters/Breeding Male</strong></td>
<td>0.79 (N = 29)</td>
<td>3     (N = 12)</td>
</tr>
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</table>

1 Total number of kits produced relative to the total number of females in the population.

An experiment to determine if Columbia Basin and Idaho pygmy rabbits could interbreed was initiated in 2003, with the successful birth of 10 intercross animals. In 2004 and 2005, intercross animals were successfully able to backcross with Columbia Basin pygmy rabbits. In April 2006, the captive population consisted of 9 rabbits that are 50% Columbia Basin, 3 rabbits that are 62.5% Columbia Basin, 47 that are 75% Columbia Basin, 6 that are 87.5% Columbia Basin as well as the 3 that are 100% Columbia Basin pygmy rabbits.

Reproductive behavior was intensively monitored at Washington State University in 2003 - 2004. Reproductive behavior showed significant differences between Columbia Basin and Idaho pygmy rabbits. Although identified behaviors were similar, Columbia Basin pygmy rabbits spent 8 times longer (averaging 964 minutes) than Idaho pygmy rabbits (124 minutes) before
beginning reproductive behavior. Columbia Basin pygmy rabbits spent 5 times longer chasing/hour once reproductive behavior began than Idaho rabbits, however both groups had equal numbers of mountings (Elias 2004). In addition, in two years of breeding, Columbia Basin pygmy rabbits did not successfully breed after April 12, while Idaho pygmy rabbits successfully bred through May and into early June.

The poor reproductive performance of Columbia Basin pygmy rabbits compared to Idaho pygmy rabbits endangers the continued existence of Columbia Basin pygmy rabbits in captivity, and has prevented the production of sufficient number of animals to reintroduce back into the wild. The size of the Columbia Basin captive population to date has been declining. Projections using population modeling software PM 2000 indicate continued declines in Columbia Basin pygmy rabbits. Intercross animals, however, have markedly increased fitness (as exhibited by reproductive success) over the Columbia Basin pygmy rabbits.

Medical Issues

Pygmy rabbits have been maintained in captivity on soil. The two principal disease issues of concern for captive pygmy rabbits have been coccidiosis and mycobacteriosis, both related to soil relationship. Disseminated mycobacteriosis due to Mycobacterium avium has been the most common cause of death of adult captive pygmy rabbits. Fatal disseminated mycobacteriosis due to M. avium was diagnosed in 28 captive pygmy rabbits between June 2002 and September 2004, with deaths occurring at 2 of the 3 captive holding facilities. M. avium is a bacterium that commonly exists in soil and water, and can survive for long periods of time in soil. High numbers of the bacterium can be shed in feces. The incubation period of mycobacteriosis can be weeks to months, and detection of infected individuals is difficult. Treatment can take several years and its efficacy in pygmy rabbits is unknown. There may be side-effects of treatment, including reduced reproductive performance. Currently, regular fecal cultures are done on captive rabbits in an attempt to detect subclinically infected animals.

Antemortem and postmortem medical records from these cases were evaluated retrospectively with the goals of describing the clinical behavior of mycobacteriosis in pygmy rabbits (Harrenstien et al. unpubl manuscript). We assessed the relative value of physical examination findings and diagnostic test results in the presumptive or definitive diagnosis of mycobacteriosis in pygmy rabbits, use of various treatment protocols, possible risk factors for the observed high mortality, and developed recommendations for prevention of mycobacteriosis. Partially-ineffective cell-mediated immunity appears to be the strongest reason for the high morbidity and mortality of M. avium infections in pygmy rabbits.

Coccidiosis is caused by a protozoan that invades the intestines and other tissues of animals. A new pathogenic species of coccidian, Eimeria brachylagus has been identified from captive pygmy rabbits (Duszynski et al. unpubl manuscript). Four captive-born Columbia Basin pygmy rabbit young and 3 captive-born Idaho pygmy rabbit young died of intestinal coccidiosis during the 2002 breeding season. In 2003, four captive-born Columbia Basin pygmy rabbit young and at least 6 young Idaho pygmy rabbits died of intestinal coccidiosis. The species of coccidia infecting pygmy rabbits is under study, but has not been positively identified. Antibiotics have been effective at decreasing parasite loads. Since the deaths in spring 2002, coccidia levels are now monitored in captive pygmy rabbits. Animals with elevated coccidia levels are treated with antibiotics.
The heavy mycobacteriosis losses in the captive Columbia Basin pygmy rabbits have caused the building of 30 experimental non-soil pens for animal husbandry. Animals will be kept off soil until the breeding season, unless we develop successful reproduction off-soil.

The high incidence of mycobacteriosis led the Oregon Zoo to initiate an investigation into the rabbits' cellular immune function. In collaboration with researchers from the National Institutes of Health, blood samples from 14 Columbia Basin pygmy rabbits and 9 Idaho pygmy rabbits were tested to determine the response of their immune system cells to infection. In general, Columbia Basin pygmy rabbits had a significantly poorer immune response than the Idaho pygmy rabbits (K. Mansfield, WDFW, pers. comm.). While at this point it is not possible to say with certainty whether this finding is related to the higher degree of inbreeding in Columbia Basin pygmy rabbits, a relationship between diminished genetic diversity and higher susceptibility to mycobacteriosis has been demonstrated for a number of other species.

A new mortality factor emerged during the summer of 2004. Approximately 14 pygmy rabbits housed at Washington State University have died of an intestinal disease of unknown origin. All genetic lines (Columbia Basin, Idaho, and intercross) have been affected. Most cases have been young adults, although some young of the year have also died. As of March 1, 2005, no cases have been detected at the other two captive breeding facilities, the Oregon Zoo and Northwest Trek. A decision was made to suspend plans to move rabbits between facilities. This suspension will result in fewer desired pairings than would have occurred with animal exchange, but is being taken to ensure this disease does not spread to other facilities.

Genetic Management

Conservation Genetic Principles used in Captive Breeding Programs

Most captive breeding programs for endangered species are designed to maintain a specific percentage of wild populations' genetic diversity for a specified time period. Soulé et al (1986) originally proposed that there should be an effort to retain 90% of the wild population’s genetic diversity for a period of 200 years, although in practice, this goal has been relaxed to a lower percentage for a shorter period of time. Attempts to retain in captivity a certain percentage of the wild population’s genetic diversity are grounded in two fundamental assumptions: (1) the wild population is still relatively large (e.g., Ne [effective population] > 500, or N > 1000 individuals) and genetically diverse, and (2) the founding population for the captive program is a random sample of the wild population, and is large enough to retain most of the genetic diversity (measured as allelic diversity or heterozygosity) of the wild populations (see Frankham et al. 2002 for discussion of specific goals).

In order to retain a significant proportion of the wild population’s genetic diversity, captive-breeding managers must attempt to maximize the size of the captive population, given the limits imposed by the breeding facilities. This is because the deterioration of genetic diversity as a result of genetic drift (loss of alleles) and inbreeding (loss of heterozygosity) is reduced in larger versus smaller populations. There is also a need to maximize Ne/N, in order to maximize genetic diversity per size of the breeding population.
In addition to retaining genetic diversity, captive breeding programs must also attempt to avoid inbreeding depression (reduction in survival or reproduction as a result of inbreeding) or the accumulation of deleterious mutations (e.g., producing homozygous individuals for recessive alleles that will reduce fitness in its homozygous state). Managers designing breeding programs can minimize these affects by pairing captive individuals with low kinship values. This will potentially avoid the negative factors associated with inbreeding depression and help maintain a level of genetic diversity by preserving heterozygosity within the captive population.

Summary of the population genetics of the captive-bred Columbian Basin Pygmy Rabbits

Methods
Twelve microsatellite loci developed from European rabbit (*Oryctolagus cuniculus*) were screened for amplification and variability in pygmy rabbits. Of these 12 loci, nine were selected for use in this analysis (Table 1). Eight of these microsatellite loci have di-nucleotide repeat sequences, and one locus has a tetra-nucleotide repeat sequence. PCR protocols for these loci were modified from those in the original publications (Table 2) to facilitate multiplexing and amplification in pygmy rabbits. Protocols are available from author upon request. All PCR amplifications were conducted with MJ Research PTC-200 thermocyclers using fluorescently labeled primers (oligonucleotide primers synthesized by Integrated DNA Technologies, Inc. [IDT] or Applied Biosystems [ABI]). DNA fragments were visualized using an Applied Biosystems ABI Prism 377 sequencer or a 3100 Genetic Analyzer, and sized using an internal lane/capillary size standard (Applied Biosystems GeneScan 500 – ROX), and ABI GeneScan 3.7 and ABI Genotyper 3.7 software running on MS Windows NT/2000-based computers. We calculated allele frequencies, expected heterozygosity, observed heterozygosity, allelic richness, and other measures of genotypic diversity using Fstat (Goudet 2001), GDA (Lewis and Zaykin 2001), or Microsatellite Analyzer (MSA; Dieringer and Schlötterer 2003).

Founders
Sixteen Columbia Basin pygmy rabbits were trapped at Sagebrush Flats, Washington and transported to captive breeding facilities at the Oregon Zoo (OZ) and Washington State University (WSU) between May 7, 2001 and January 15, 2002 (Figure 1). These 16 rabbits (Founders) represented an unknown but presumably sizeable portion of the then-existing population of Columbia Basin pygmy rabbits. No other Columbia Basin pygmy rabbits have been captured since January 2002, despite repeated attempts. None of the original 16 rabbits that formed the founding population are now alive, and unless new pygmy rabbit populations are discovered, the Columbia Basin rabbits may now be extinct in the wild. Therefore, the genetic diversity of the Founders, designated as CB2001 in Table 2, represents not only the initial genetic conditions for the captive breeding program, but also perhaps the genetic diversity of the entire population of Columbia Basin pygmy rabbits in 2001. We compared the genetic diversity of CB2001 to that from a population of Idaho pygmy rabbits trapped one year earlier (20 June 2000 through 21 December 2000) from Lemhi County, Idaho (IDWILD). For each measure of genetic diversity (Table 2) the Idaho population is approximately two-times as diverse as the Columbia Basin population (CB2001), despite the fact that the sample sizes are roughly equal and the geographic extent from which the samples were drawn is the same (Figure 2).

The genetic diversity of the Founders can also be compared temporally with two historical samples from Sagebrush Flats: a museum skin collection from 1949 and 1950, and a blood
sample collected by Gahr (1993) in 1992. All three Columbia Basin samples show lower genetic diversity than the Idaho samples, and there appears to be a decline in genetic diversity from 1949 to 2001 (Table 2). The captive breeding program for Columbia Basin pygmy rabbits was initiated with a population that showed the lowest genetic diversity of all wild populations sampled thus far, and from a population that appears to have been declining in genetic diversity for over 50 years. Because we brought all known Columbia Basin individuals into captivity, we suspect we captured the remaining genetic diversity within the wild Columbia Basin pygmy rabbits.

Captive Breeding

The founding population was split into two “subpopulations” one each at the Oregon Zoo (OZ) and Washington State University (WSU), and was immediately augmented by five kits from Danae, who was bred in the wild (i.e., father of the kits is unknown; Figure 1). The nine microsatellite genotypes for each of these kits were identical and differed from Danae at only two of the 18 alleles, suggesting that Danae bred with an individual with nearly the same genotype as her, possibly a close relative. The survivors from the Founders and Danae’s offspring were the individuals that constituted the initial captive breeding population (CB2002; Table 2, Figure 1). We defined each captive population based on those named individuals alive at the beginning of each calendar year, and calculated the theoretical relatedness (sensu Queller and Goodnight 1989) between each pairwise combination of males and females. Breeding pairs were selected initially (2001-2004) based on individuals with the lowest pairwise relatedness, and priority was given to the most genetically diverse individuals (i.e., possessed rare alleles). Beginning in 2005, we based our breeding strategy on the kinship values calculated from the actual pedigrees, using PM2000 (Pollak et al., 2005). Although the captive population exists at two localities (OZ and WSU) it is managed as a single population, with individual rabbits being moved between localities in order to achieve the most optimal pairings.

There have been four captive breeding seasons for the Columbia Basin pygmy rabbits. As defined by Figures 1 and 2, and Table 2, the 2002 population produced the 2003 population, the 2003 population produced the 2004 population, and so on, resulting in the current 2006 population (CB2006-mix; Table 2). Not included in the 2003 Columbia Basin population, but participating in an experimental Columbia Basin – Idaho crossbreeding experiment in 2003 were two males from the Idaho National Engineering and Environmental Laboratory (INEEL) population (Alder and Behlem), who are shown in Figure 2. The population that included all the Columbia Basin individuals alive in January 2004 and offspring from the Columbia Basin – Idaho INEEL intercross (not including pure IDINEEL individuals) is represented as the CB2004-Mixed population (Table 2). From this point forward (i.e., 2004-2006), we represent populations as either pure Columbia Basin (e.g., CB2006-CB) or Columbia Basin plus intercross individuals (e.g., CB2006-Mixed). For each population pair (e.g., CB2006-CB and CB2006-Mixed), the genetic diversity is greater for the mixed population, and for most years, when considering the mixed populations, there has been an increase in genetic diversity from 2003 to 2006 (Table 2; Figure 4). This is opposite to the 2003-2006 trend when considering the pure Columbia Basin populations (Table 2; Figure 4).

Gene Diversity in the Captive Pygmy Rabbit Population

Table 3 provides a summary of gene diversity statistics for historical wild populations of Columbia Basin pygmy rabbits, and for the two captive populations of pygmy rabbits, as defined
above. The *genetic* gene diversity statistic is the expected heterozygosity calculated from the nine microsatellites loci. The *pedigree* gene diversity statistic is from PM2000 and represents the proportional change in gene diversity from the founding population to the current population. Although PM 2000 considers this gene diversity to be related to the expected heterozygosity, it is calculated directly from the pedigree as 1-MK (MK = mean kinship\(^1\) of all nonfounders in the current population). The current genetic gene diversity (expected heterozygosity) for the Columbia Basin population is 0.27, which is a 33% - 40% decline from the historical expected heterozygosity at Sagebrush Flats (SBF). The pedigree gene diversity (gene diversity) for the captive pure Columbia Basin population equals 85% of the founders' gene diversity (Table 3); this represents an increase in mean kinship of 15% in just five years.

As expected, the gene diversity of the non-Idaho or mixed population is greater than that for the pure Columbia Basin rabbits for both the genetic and pedigree statistics (Table 3). In fact, the expected heterozygosity for the mixed population (0.56) is 24% greater than the most diverse historical population of Columbia Basin rabbits (SBF-1949), and nearly 107% greater than the pure Columbia Basin rabbits in captivity (populations are not independent in that the pure Columbia Basin rabbits are a major component of the mixed population). The increase in genetic diversity is a function of the addition to the Columbia Basin captive breeding program of descendents from seven pure Idaho pygmy rabbits through the intercross matings (see below). Although the expected heterozygosity has increased with the addition of the intercross rabbits, the pedigree-based gene diversity is 0.91 (Table 3) and therefore indicates an 9% decline in gene diversity from the founding population\(^2\). The reason the pedigree-based statistic shows a decline in gene diversity is because the effective population size is less than the census population size. That is, not all adult individuals have bred with equal frequency, and the number of offspring produced by these breeding adults is not equal. These factors reduce the effective population size below that of the census population and therefore increase mean kinship among the surviving individuals in the population.

**Founders Input to Captive Population**

There are a total of 15 founders in the mixed population; eight founders from Columbia Basin, and seven from Idaho, as discussed above. These founders have not contributed equally to the current mixed population (Table 4). The founders' input to the captive population can be represented using any one of four statistics: representation\(^3\), contribution\(^4\), allele retention\(^5\), and number of descendants (calculated in PM2000). We have also added a fifth statistic (PCA1), which is the weighted linear combination of each of the four PM2000 statistics that account for the most total variance in the data set (i.e., the first principle component, using a total correlation matrix). These five statistics are shown in Table 4. Although three founders each have greater

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\(^1\) It is important to differentiate "kinship" from relatedness. Kinship is the probability that two randomly chosen alleles, one from each of two individuals, are identical by descent (Blouin, 2003), and can be calculated directly from a pedigree without specific knowledge of the any particular locus. Relatedness is the proportion of alleles in two individuals that are identical by descent (Blouin, 2003). Relatedness can be calculated from genotypic data or as 2 times kinship.

\(^2\) Founding population for the mixed population includes all the Columbia Basin Idaho founders

\(^3\) The proportion of the alleles in the living captive population that are derived from the founder. This is the proportional founder contribution.

\(^4\) The number of copies of the founder's genome present in the living captive population (e.g., offspring = 0.50, grand offspring = 0.25).

\(^5\) The probability that an allele present in the founder exists in the living captive population.
than 60 descendants, the most among all founders, Elymus (60 descendants) has had the greatest input into the captive population, as measured by PCA1, which summarizes the other four statistics. All founders are now dead (Elymus died March 30, 2006), and changes to their contribution to the captive population will be a function of breeding performance of their descendants. As expected, Columbia Basin input into the mixed captive population is greater than that of the Idaho founders, based on all founder statistics. For example, there are total of 82 "genomes" present in the living captive population (see Contribution), of which 75% are Columbia Basin genomes. Likewise, there are 519 descendants from the 15 founders in the mixed captive population, with 395 or 76% from Columbia Basin rabbits. Among the Columbia Basin rabbits, each male founder has averaged a greater input to the living captive population compared with each female founder. Fifty nine percent of the Columbia Basin contribution, and 57% of the Columbia Basin descendants are from male rabbits, and the allelic retention for males is 43% greater than that for females. Among the Idaho rabbits, Alder has been most successful, leaving 50 descendants and 10.75 genomes (Contribution). His allelic retention is as larger than all Columbia Basin rabbits, except Bam Bam.

**Genetic Goals for the Captive Population of Pygmy Rabbits**

Over the past four years, the captive breeding program for the pure Columbia Basin pygmy rabbits has maintained 73% - 95% of the genetic diversity of the founding population (Table 2). However, the captive breeding program of pure Columbia Basin pygmy rabbits is vulnerable because (1) only three pure Columbia Basin pygmy rabbit remain alive, as of April 15, 2006; (2) the genetic diversity of the Columbia Basin founding population was low; (3) the effective population size of the captive populations is low (Ne = 5.7; population on January 1, 2006) and therefore genetic drift may further decrease genetic diversity, and (4) there are no known wild Columbia Basin pygmy rabbits available to genetically augment the captive breeding program. Therefore the primary genetic goals of the Columbia Basin captive breeding program are as follows:

1. Increase the effective size (Ne) of the population by increasing the number of breeding adults participating in the breeding program.
2. Increase the census size of the captive population by searching for and removing additional wild Columbia Basin pygmy rabbits.
3. Infuse new genetic diversity into the Columbia Basin population by continuing an intercross strategy to reverse the negative effects of genetic drift and inbreeding. This action will dramatically increase the level of genetic diversity within the population (Table 2, Figure 4), and lessen the affects of genetic drift by increasing the effective population size.

**2006 Management Priorities**

Given the above discussion, the 2006 management priorities for the captive breeding program are:

a) Maintain genetic diversity of Columbia Basin Pygmy rabbits by matching the most appropriate pure Columbia Basin males and females (minimize loss of genetic diversity),
b) Produce offspring of pure-bred Columbia Basin pygmy rabbits,
c) Conduct intercrosses to produce 87.5% Columbia Basin pygmy rabbits
d) De-emphasize breeding of rabbits with a high relatedness to the Idaho rabbit Alder(See Table 4).
e) Conduct intercrosses to produce 75% Columbia Basin pygmy rabbits
f) Create no new F1 intercrosses (Columbia Basin/Idaho intercrosses) and avoid pairings to produce 62.5% Columbia Basin pygmy rabbits.
g) Conduct breeding experiments to compare results of large pen breeding to small pen breeding
h) Conduct experimental off-soil breeding.

Objective A) Maintain genetic diversity of Columbia Basin Pygmy rabbits by matching the most appropriate Columbia Basin males and females (minimize loss of genetic diversity).

This objective is achieved through analysis of the rabbit pedigree, genotyping all individuals, and prioritizing specific pairings. Genetic relatedness, both theoretical based upon allelic diversity, and kinship based on the pedigree have been used to determine pairings. For 2006 we will kinship values calculated in PM2000, and founder statistics to prioritize breeding pairings.

Objective B) Produce offspring of 100% Columbia Basin pygmy rabbits.

Pairings of Columbia Basin pygmy rabbits will be the highest priority from March 1 through April 15. This period includes all known dates of successful Columbia Basin pairings from 2002 through 2005 with a buffer of one additional week. However, in 2006, we have few healthy Columbia Basin individuals to pair. The most important individual is Raphaela at WSU, and she will be primarily paired with Heath for this period. The other important pairing will be Lolo and Bryn at the Oregon Zoo.

Objective C) Conduct intercrosses breeding to produce 87.5% or higher Columbia Basin pygmy rabbits.

Intercrosses that are 75% or 87.5% Columbia Basin pygmy rabbits will be bred with 100% Columbia Basin pygmy rabbits.

Objective D) De-emphasize breeding of rabbits with a high kinship to the Idaho rabbit Alder.

There are currently 28 animals that are in the captive breeding pool that have relatively high kinship to the Idaho rabbit Alder. To de-emphasize Alder in the captive population we will take a number of steps. First we will not breed the F1 (50%) Columbia Basin male Ivan. We will also only breed the 3 50% female pygmy rabbits Gretchen, Wolfgang, and Inge with 100% Columbia Basin males. In addition, the 11 males (Watson, Crick, Starsky, Buckley, Pan, Orlando, Vigo, Kerr, Periwinkle, Onyx, and Moore) that have relatively high kinship to Alder will be de-emphasized in the captive breeding program. This de-emphasis will be done in several different ways. First they will not be bred in large pens, as we anticipate males in large pens contributing disproportionately to males bred in small pens. Second, we will use one or more of these males for non-soil breeding experiments. Third, we will attempt to reduce the number of breeding introductions (introduced pairings) of the remaining males with high kinship to Alder through careful breeding management. This means that other males will have priority in breeding pairings wherever possible.
Objective E) Conduct intercrosses to produce 75% Columbia Basin pygmy rabbits

Consistent with our de-emphasis with animals significantly related to Alder, we will emphasize the production of 75% Columbia Basin pygmy rabbits in 2006. This will be accomplished with pairings of 100 and 50% Columbia Basin animals, 75% with 75% Columbia Basin animals, and 87.5% with 62.5% Columbia Basin animals.

Objective F) Conduct research into artificial insemination.

The poor reproductive performance of Columbia Basin pygmy rabbits resulted in several genetically important animals dying without passing on their genes to offspring. The purpose of this research is to develop artificial insemination methods to maintain genetic diversity within the captive Columbia Basin pygmy rabbits through human intervention. In 2004 we attempted electroejaculation on 4 separate occasions with 7 males (all were Idaho animals). Rabbits were anesthetized by inhalation of isoflurane with oxygen via box induction. Males initially responded to electro-stimulation with muscle tension, hind limb extension and penile erection however we were unable to collect a viable semen sample during any of these attempts. Males generally showed the best response at the onset of stimulation or immediately following rest periods and rapidly became refractory to further stimulation. In one case we did collect a small volume of seminal fluid (~20 μl) which contained a few non-motile spermatozoa. We also attempted this procedure on 2 domestic rabbits (silver foxes weighing ~ 3.5 kg) and collected viable semen samples from both males. Differences in anesthetic depth between the pygmy rabbits and the domestics may partly explain these results. In the future we may wish to attempt electroejaculation of pygmy rabbits at a shallower anesthetic plane or without general anesthesia.

Fecal testosterone extraction and measurement was performed by radioimmunoassay. Mean weekly fecal testosterone levels did not differ significantly between ID and CB populations for the one month sampled during the breeding season (3/28/04-4/28/04). However, 2 out of the 4 CB males sampled did show significantly lower mean weekly fecal testosterone levels compared to all other males from either population. After the end of the breeding season (5/24/04-6/25/04), ID males maintained significantly higher weekly fecal testosterone levels in each of the 4 weeks sampled. Individual, weekly fecal testosterone level during the breeding season was not correlated with breeding success during the entire 2004 season (defined as the ratio of litters sired to pairings). Buck age was a predictor of breeding success (r²=0.789). 2 year old males were more successful than either 1 or 3 year olds.

Objective G) Conduct Experimental off-soil breeding.

Principal mortality factors for pygmy rabbits in captivity have been coccidiosis and mycobacteriosis. Both of these micro-organisms are contained in and passed to pygmy rabbits through soil. We built new pens for pygmy rabbits with concrete floors that could be adapted to either with soil or without soil. Four female Idaho rabbits and 2 male Idaho rabbits were used at WSU in 2004 to test the effectiveness of breeding pygmy rabbits off soil. Both males and females were housed in pens with rubber flooring and a set of nestboxes for housing. After each pairing another set of nestboxes filled with timothy hay were added to the pen to encourage nesting for birth of the kits. Six of 9 (67%) of pairings resulted in a pregnancy. Of these 6 pregnancies, only 1 lead to a successful weaning of any kits, but only after the female was
transferred to a soil pen to give birth. All other successful pregnancies resulted in the kits dying within 4-5 days after birth. A total of 10 kits were known to have been born (carcasses found; other carcasses were consumed by the dam), of which 2 survived past weaning. In 2006, we will continue to experiment with off-soil breeding and selective introduction of soil into the breeding process.

**Capacity of Breeding Facilities During 2006**

Currently, we have 70 permanent pens for pygmy rabbits between Washington State University, the Oregon Zoo and Northwest Trek Wildlife Park, 30 of these available for non-soil care. Each pen holds one adult animal or one litter of kits (temporarily). We also have 7 large pens of 600 to 800 sq. ft. each at Washington State University for experimental breed in large enclosures. Washington State University has a pre-release pen that can be re-furbished for animals to be released to the wild. Smaller pens are located at all three facilities, including WSU’s pre-release pens. We can temporarily hold an additional 30 – 50 animals in these areas for approximately six months. In 2006 we will build temporary pens at all three facilities to hold animals that will likely be released.

**Literature Cited**


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**Table 1.** Microsatellite data used in this analysis

<table>
<thead>
<tr>
<th>Name</th>
<th>Species of Origin</th>
<th>Repeat Sequence</th>
<th>Reference</th>
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<tbody>
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<td>sat5</td>
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<td>(TC)$<em>{23}$ TTT (CT)$</em>{5}$</td>
<td>Mougel, et al. (1997)</td>
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<td>Species</td>
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<td>(GT)$_{17}$</td>
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TABLE 2. Genetic diversity measures for 12 spatial or temporal populations of pygmy rabbits in Idaho (ID) and Washington (Columbia Basin, CB). The IDWILD population is from Lemhi County, Idaho. All Columbia Basin populations are from Sagebrush Flats. The Columbia Basin populations identified as “-CB” include only pure, 100% Columbia Basin rabbits, while those populations identified as “-Mixed” include living rabbits ≥ 50% Columbia Basin. See text.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>A</th>
<th>A_Richness</th>
<th>H_e</th>
<th>H_o</th>
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<tr>
<td>IDWILD</td>
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<td>5.84</td>
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<td>CB1949</td>
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<td>5.78</td>
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N = mean sample size per locus
A = mean number of alleles per locus
A_Richness = allelic richness (mean number of alleles per locus, standardized across all population due the sample with the smallest sample size (n=9; see El Mousadik and Petit 1996)
H_e = Gene Diversity
H_o = observed heterozygosity
**Table 3.** Gene diversities for historical populations at Sagebrush Flats, and for two captive populations, as defined in the text.

<table>
<thead>
<tr>
<th>Population</th>
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<tr>
<td>SBF-1949</td>
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<td>SBF-1992</td>
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<tr>
<td>SBF-2001</td>
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<tr>
<td>SBF.1948-2001</td>
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<td>Captive-Alive.Pure Columbia Basin</td>
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</tr>
<tr>
<td>Captive-Alive.Non-Idaho Population</td>
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<tr>
<td>Name</td>
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<tr>
<td>-----------------------</td>
<td>----------</td>
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<tr>
<td>Elymus</td>
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<td>Alder</td>
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<td>Bam Bam</td>
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<tr>
<td>Danae</td>
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<tr>
<td>Wild (Danae's mate)</td>
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<td>Allie</td>
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<tr>
<td>Timothy</td>
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</tbody>
</table>

**Columbia Basin**

- Male: 0.75, 61.25, 0.80, 395.00
- Female: 0.44, 36.34, 0.87, 225.00

**Idaho**

- 0.25, 20.75, 0.50, 124.00
FIGURE 1. The first three populations of Columbia Basin pygmy rabbit captive breeding program. Except for the Founders population and parents with extant offspring, only those individuals alive at the beginning of the year that designates the population are included in the figure. Dead offspring are represented by filled squares (males) or circles (females). Males are within blue boxes, females within red boxes. Dates associated with each of the Founders are the capture dates.
Figure 2. The 2004 Columbia Basin pygmy rabbit population in captivity at WSU and OZ. This population represents the first population with intercross animals. Alder and Behlem labeled as (ID) are pure IDINEEL individuals. See Figure 1.
**Figure 3.** The absolute distance between an individual sample and the centroid for all samples within a single locality for the Washington (Sagebrush Flat), and Idaho (IDWILD; Lemhi County) localities. Distances were calculated using UTM coordinates for each sample, where available (Idaho), or from initial x-y telemetry coordinates (Washington; see Gahr, 1993).
FIGURE 4. Allelic Richness and Gene Diversity statistics for the eight Columbia Basin populations described in the text and listed in Table 2. These graphs are intended to show two points: (1) the decline in genetic diversity of pure Columbia Basin rabbits; and (2) the dramatic increase in genetic diversity when intercross, or mixed animals are included in the population. See Table 2.