No Inbreeding Depression Observed in Mexican and Red Wolf Captive Breeding Programs

STEVEN T. KALINOWSKI,* PHILIP W. HEDRICK,† AND PHILIP S. MILLER‡

*Conservation Biology Division, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112, U.S.A., email steven.kalinowski@noaa.gov
†Department of Biology, Arizona State University, Tempe, AZ 85287–1501, U.S.A.
‡Conservation Breeding Specialist Group, 12101 Johnny Cake Ridge Road, Apple Valley, MN 55124–8151, U.S.A.

Abstract: *Inbreeding depression is expected to affect populations of outbreeding mammals in inverse proportion to their population size and can affect whether small populations persist or go extinct. We used studbook records to examine the effect of inbreeding upon juvenile viability and litter size in two endangered species that have recently been reintroduced to the wild: the Mexican wolf (Canis lupus baileyi) and the red wolf (C. rufus). We found that neither juvenile viability nor litter size was lowered by inbreeding in either taxon. In fact, both captive breeding programs appear to have less lethal equivalents than the median estimate for mammals. We did find that year of birth was correlated with increasing viability in both taxa. We conclude that there is no evidence that inbreeding depression will prove a major obstacle to the success of either recovery effort.*

Depresión en el Lobo Rojo y el Lobo Mexicano no Ocasionada por Intracruza en los Programas de Reproducción en Cautiverio

Resumen: *Se espera que la depresión por intracruza afecte poblaciones de mamíferos en proporción inversa a su tamaño poblacional y podría determinar la persistencia o extinción de una población. Utilizamos libros de registro para examinar el efecto de la intracruza en la viabilidad de juveniles y tamaño de camada en dos especies amenazadas que han sido recientemente reintroducidas en áreas silvestres: el lobo Mexicano (Canis lupus baileyi) y el lobo rojo (C. rufus). Encontramos que ni la viabilidad de juveniles, ni el tamaño de camada fueron reducidos por la intracruza en alguno de los taxones. De hecho, ambos programmas de reproducción en cautiverio aparentan tener menos equivalentes letales que la mediana estimada para mamíferos. Encontramos que el año de nacimiento estuvo correlacionado con un incremento en la viabilidad de ambos grupos. Concluímos que no existe evidencia de que la depresión por intracruza puede ser un obstáculo mayor en el éxito de los esfuerzos de recuperación.*

Introduction

In a classic conservation biology study, Ralls et al. (1988) showed that 36 out of 40 zoo populations exhibited decreased juvenile viability in inbred individuals. The median estimate of the number of lethal equivalents in a diploid genome was 3.14, but there was extensive variation among estimates, including populations with nonsignificant inbreeding depression and increased viability among inbred individuals. Subsequent research has

found inbreeding depression among many traits, with variation occurring across species, populations, lineages, and individuals (e.g., Pray & Goodnight 1995; Kärkkäinen et al. 1996; Lacy et al. 1996). Recognition of this potential effect has made inbreeding depression a concern in small-population conservation and inbreeding avoidance a priority in captive breeding programs.

Two endangered wolf species, the Mexican wolf (*Canis lupus baileyi*) and the red wolf (*C. rufus*), were almost extinct when the last wild members of the species were captured in order to start captive breeding programs. Both species have now been reintroduced to the wild and are being monitored closely. Although many as-

1371

pects of wolf biology will affect whether these populations grow, the genetic health of the released wolves is among the most fundamental. Quantifying the hidden deleterious alleles within these species, by examining the impact of inbreeding upon fitness, may provide insight to the outcome of these recovery efforts. Inbreeding has been demonstrated to decrease growth rate and cause blindness in captive Fenno-Scandian wolves (*C. lupus*; Laikre et al. 1993) and has been a concern for the small population of wolves on Isle Royale (e.g., Wayne et al. 1991). Critics of the Mexican wolf recovery effort have publicly argued that inbreeding will prevent successful recovery of the population. In contrast, many wolf experts seem to believe that inbreeding in the wild has purged many of the deleterious alleles from wolf populations.

The Mexican wolf is a subspecies of the gray wolf that once occupied the southwestern United States and northern Mexico. Mexican wolves were eliminated from the wild in the United States approximately 30 years ago and are believed extinct in Mexico (there has been no authenticated information on wild wolves in Mexico for over a decade). A U.S. Fish and Wildlife Service (USFWS) captive breeding program began with wolves captured in Mexico between 1976 and 1980, of which three have descendants in the present population (Hedrick et al. 1997). The breeding program avoided inbreeding to the extent possible, but with only three founders, inbreeding quickly became inevitable. The captive population has grown to nearly 200, and in early 1998, 11 wolves were released in eastern Arizona to establish a wild population.

The USFWS captive population of wolves has been named the certified lineage of Mexican wolves in order to differentiate it from two additional lineages of captive Mexican wolves, the Aragon and Ghost Ranch lineages, which have been raised independently. Concern that these two lineages were not pure Mexican wolves kept them from use in the USFWS breeding program until recent molecular analysis demonstrated their taxonomic integrity and led to crossings between lineages (Hedrick et al. 1997). Because the certified lineage is the largest and has the best-kept records, we restricted our analysis to it.

The red wolf once ranged across the southeastern United States from Texas to North Carolina. There has been controversy over its designation as a distinct species (see Nowak & Federoff 1998; Wayne et al. 1998, and references therein) because some red wolves have hybridized with coyotes (*C. latrans*). The last remaining red wolves were captured in Texas and Louisiana in the late 1970s. Of these, 40 did not appear to have coyote ancestry and were considered true red wolves. Only 28 individuals produced offspring, however, and 13 have descendants in the present population. With 13 founders, there was much less inbreeding in the red wolf breeding program than in that for the Mexican wolf. In 1987 the first release of red wolves was carried out in the Alligator National Wildlife refuge in North Carolina.

Methods

We obtained pedigrees, date and place of birth, and date of death from the studbooks of each wolf taxon (Waddell 1997; Siminski 1998). We calculated inbreeding coefficients from each pedigree. We fit the traditional model (Morton et al. 1956) of inbreeding's effect upon viability,

$$S_i = S_0 e^{-Bf_i}, \tag{1}$$

(Kalinowski & Hedrick 1998) to these data using survival to 180 days as criterion for viability. In this model, S_i is the expected viability of individuals with an inbreeding coefficient of f_i, S_0 is the viability of noninbred individuals, B is a measure of how fast viability decreases with inbreeding, and $2B$ is approximately the number of lethal equivalents in a diploid individual. A lethal equivalent is a unit of genetic variation, and the number of lethal equivalents within an individual is equal to the sum of the selective disadvantage of all detrimental or lethal alleles when homozygous (Cavalli-Sforza & Bodmer 1971). Morton et al.'s model assumes that loci affecting viability act independently and multiplicatively, and that each individual has the same probability of dying from nongenetic effects.

We tested the model's implicit assumption that each founder had the same number of lethal equivalents by calculating maximum likelihood estimates of the number of lethal equivalents brought to the pedigree from each founder with the model

$$S = S_0 \exp\left(-\sum_{i=1} B_i f_i\right),$$

where B_i is the number of lethal equivalents in the ith founder and f_i is the proportion of an individual's genome identical by descent with an allele from that founder (Lacy et al. 1996; Lacy 1997). Note that Σf_i taken over all founders equals the traditional inbreeding coefficient, f. We tested variability among estimates of B for each of the founders for statistical significance with a likelihood ratio test that compared two hypotheses: H_0, each founder in the pedigree has the same value of B and H_A, each founder in the pedigree has a unique value of B. The likelihood of each hypothesis was calculated analogous to the model presented by Kalinowski and Hedrick (1998).

We were interested in examining whether wolves born during different years had the same chance of surviving to 180 days, independent of the effects of inbreeding. So we examined whether S_0 in equation 1 remained constant or changed (particularly increased) with time. This is important because improvements in husbandry may obscure inbreeding depression, especially if inbreeding is correlated with time. To perform this analysis we used a logistic relationship between

year of birth (YOB) and survivorship, in combination with the exponential decline in fitness of the model of Morton et al. (1956):

$$S = S_0 \exp\left(-\sum_{i=1} B_i f_i\right), \qquad (2)$$

where

$$S_0 = \frac{e^{a+b\text{YOB}}}{1 + e^{a+b\text{YOB}}}.$$

This model is similar to the purely logistic model of Ballou (1997).

Next, we examined whether litter size was affected by maternal inbreeding or year of birth using a nonparametric test of regression similar to the Mantel test (Sokal & Rohlf 1995). For each pair of variables, we calculated the observed sample-size weighted regression coefficient. Then we randomized the data 20,000 times and recorded the fraction of regression coefficients for the randomized data as different from zero as the observed value. This fraction represents the probability of obtaining the observed regression coefficient given no relationship between the two variables. We also looked for an association between litter size and viability in litters. Lastly, we looked for variation in viability among zoos.

Results

In the certified lineage of the Mexican wolf captive breeding program, 251 individuals were born in 61 litters through 1997. In the red wolf captive breeding program, there were 688 births in 157 litters through 1996. The Mexican wolf breeding program initially had low numbers of births, but in preparation for the release, the number of births increased. The number of red wolves

Table 1. The number of litters (N_{litters}), the number of individuals born (N_{ind}), and the number of individuals surviving 180 days (N_s) with the inbreeding coefficient f, for the Mexican and red wolf captive breeding programs.

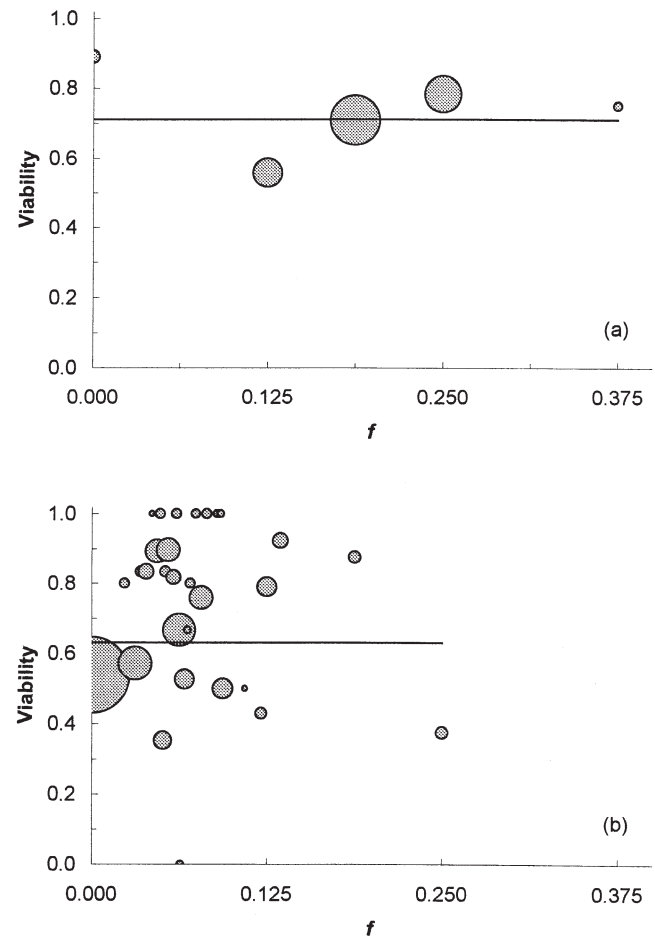|	Mexican wolves			Red wolves		
f	$N_{litters}$	N_{ind}	N_s	$N_{litters}$	N_{ind}	N_s
0.0000	2	9	8	61	294	158
0.0234				1	5	4
0.0313				13	56	32
0.0352				2	6	5
0.0391				3	12	10
0.0430				1	2	2
0.0469				6	28	25
0.0488				1	5	5
0.0508				3	17	6
0.0527				1	6	5
0.0547				7	29	26
0.0586				4	11	9
0.0605				1	5	5
0.0625				17	54	36
0.0635				1	3	0
0.0664				4	19	10
0.0684				1	3	2
0.0703				1	5	4
0.0742				1	4	4
0.0781				5	29	22
0.0820				1	5	5
0.0894				1	3	3
0.0918				1	3	3
0.0938				6	22	11
0.1094				1	2	1
0.1211				2	7	3
0.1250	8	43	24	4	19	15
0.1348				2	13	12
0.1875	28	123	87	1	8	7
0.2500	21	69	54	3	8	3
0.3750	1	4	3			
Unknown	1	3	0	1	5	4
Total	61	251	176	157	688	437

Figure 1. The observed average viability of each inbreeding class for (a) Mexican and (b) red wolves. The area of the circles is proportional to the number of individuals born, and the fitted line is based on the model of Morton et al. (1956).

born also increased around release time and has been low recently. One litter of three Mexican wolves had unknown paternity, as did one litter of five red wolves, but none of the wolves with unknown paternity had offspring. We excluded these two litters from analyses requiring paternity data, but included them in other analyses.

As expected, we found much more inbreeding in the Mexican wolf pedigree than the red wolf pedigree (Table 1; Fig. 1). The average inbreeding coefficient was 0.1903 for Mexican wolves and 0.0403 for red wolves. Almost all of the births in the Mexican wolf pedigree fell in three levels of inbreeding, with approximately half (49.6%) of the births having an inbreeding coefficient of 0.1875. In the red wolf pedigree, 43.1% of the births were noninbred and the rest were distributed across 29 levels of inbreeding.

No association was found between inbreeding coefficients of individuals and juvenile viability (Table 2; Fig. 1). The estimated value for $2B$ for each taxon was zero, indicating no detrimental effect of inbreeding upon viability. Confidence intervals showed that $2B$ was unlikely to be >1.68 or 0.74 for the Mexican and red wolf pedigrees, respectively (Table 2). This suggests that if the assumptions of the model are met, both captive breeding programs have less inbreeding depression than most species of captive mammals (Ralls et al. 1988).

We found no evidence for variation in the number of lethal equivalents among founders in either wolf pedigree. For the Mexican wolf, we obtained 0.20, 0.00, and 0.00 as maximum likelihood estimates for the number of lethal equivalents in the three founders, and these values had virtually the same likelihood of producing the observed data as 0.00, 0.00, and 0.00. For the red wolf, nine founders contributed alleles to loci identical by descent in descendants, and the estimated number of lethal equivalents in all founders was zero.

In both breeding programs, a logistic model of increasing noninbred viability (equation 2) fit the data significantly better than constant noninbred viability (likelihood ratio test, $p = 0.004$ and $p < 0.001$ for the Mexican and red wolf breeding programs, respectively; Fig. 2). In both wolf species, the maximum likelihood

estimate of $2B$, the number of lethal equivalents, remained 0.00 (Table 2) indicating an absence of inbreeding depression. Nevertheless, assuming a logistic relationship between year of birth and viability moderately increases the width of the confidence interval for $2B$ (Table 2). Given the rate of change of viability with year of birth or inbreeding coefficient within our data set, our results would be similar whether based on linear, logistic, or exponential models.

We found a trend toward decreased litter size with maternal inbreeding (Fig. 3), but the effect was not statistically significant for either species ($p = 0.29$ and 0.94 for the Mexican and red wolves, respectively). Average litter size decreased with time in both species at similar rates (Fig. 4), with p values of 0.32 and 0.06 (randomization test, as described above) for the Mexican and red wolves, respectively. In both breeding programs we
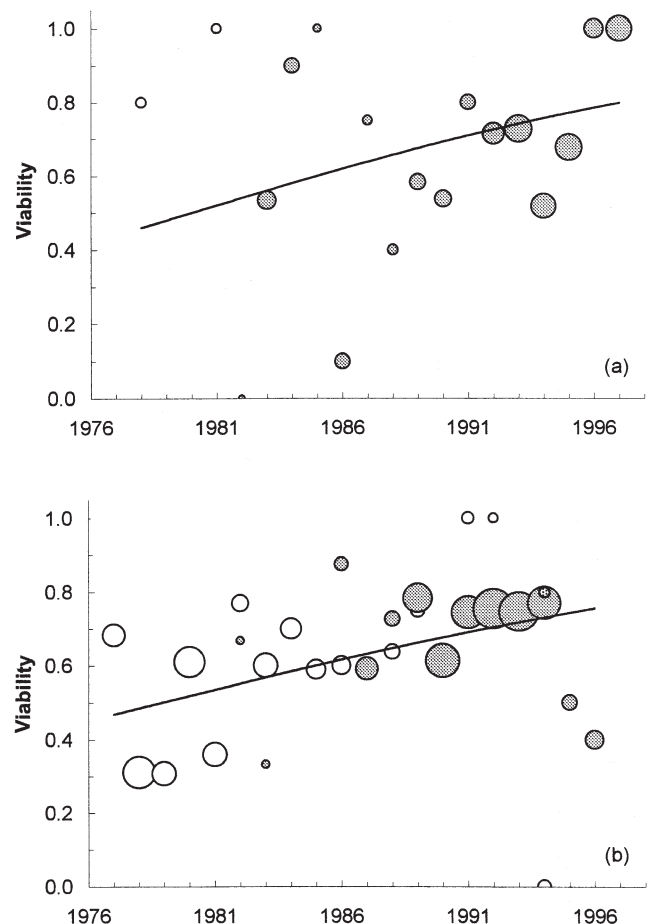

Figure 2. Observed viability of noninbred individuals (open), inbred individuals (shaded), and unknown inbreeding status (spotted circle in 1994) for the (a) Mexican and (b) red wolves. The area of circles is proportional to the number of individuals. The fitted line shows the viability of noninbred births as a logistic function of year of birth.
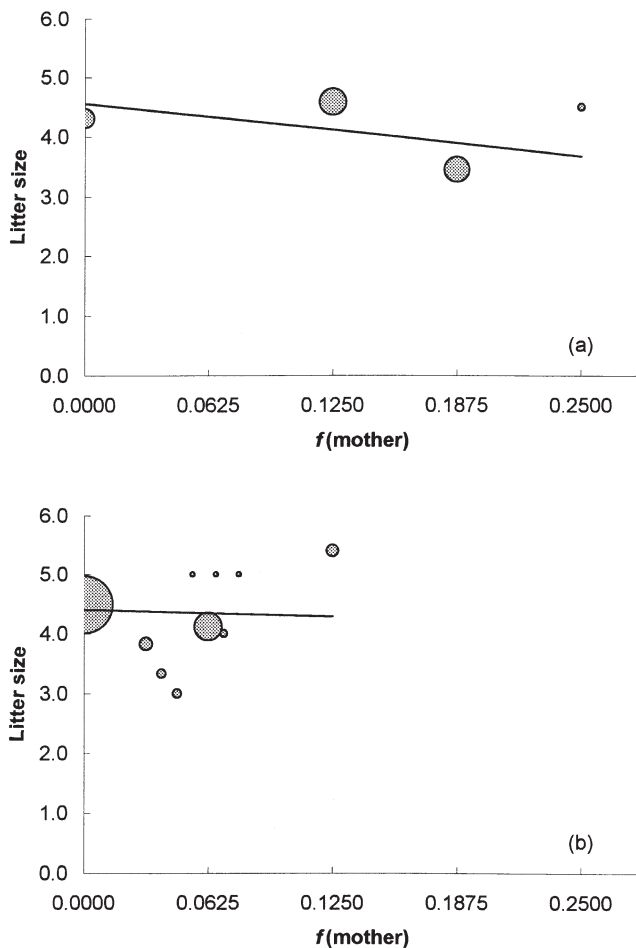
Table 2.   Maximum liklihood estimates of the viability of noninbred births, S_0, the number of lethal equivalents in the pedigree, $2B$, and 95% confidence intervals for $2B$ in the Mexican and red wolf captive breeding program using two models of viability.

		$\hat{S}_0$	
	model type	value(s)	$2\hat{B}$ (min, max)
Mexican wolf	constant	0.71	0.00 (0.00, 1.68)
Red wolf	constant	0.63	0.00 (0.00, 0.74)
Mexican wolf	logistic	from 0.46 to 0.80	0.00 (0.00, 1.87)
Red wolf	logistic	from 0.47 to 0.76	0.00 (0.00, 2.12)

Figure 3. Average litter size plotted against the in-breeding coefficient of the dam for the (a) Mexican and (b) red wolves. Fitted lines are based on litter size–weighted, least-squares linear regression, and the area of circles is proportional to the number of individuals.

Figure 4. Average litter size each year for the (a) Mexican and (b) red wolf captive breeding programs. Fitted lines are based on sample size–weighted, least-squares linear regression, and the area of circles is proportional to the number of individuals.

found no relationship between litter size and viability, with p values of 0.71 and 0.27 for the Mexican and red wolf breeding programs, respectively.

Unfortunately, the data were too sparse to permit us to examine variation in viability across zoos, especially if we accept our conclusion that year of birth affected viability. Fifteen zoos raised litters of Mexican wolves during the past 18 years, but 9 zoos did not begin breeding until 1994; over half of the wolves were born in 1993 or later. Eighty-six percent of litters were the only litter born in their respective zoo the year of their birth. Thirty-three zoos participated in raising litters of red wolves, but the Point Defiance Zoo in Tacoma was the most active. That zoo raised litters each year of the breeding program, up to seven in a year, accounting for 43% of litters. The remainder of the litters were spread out fairly evenly among the other 32 zoos.
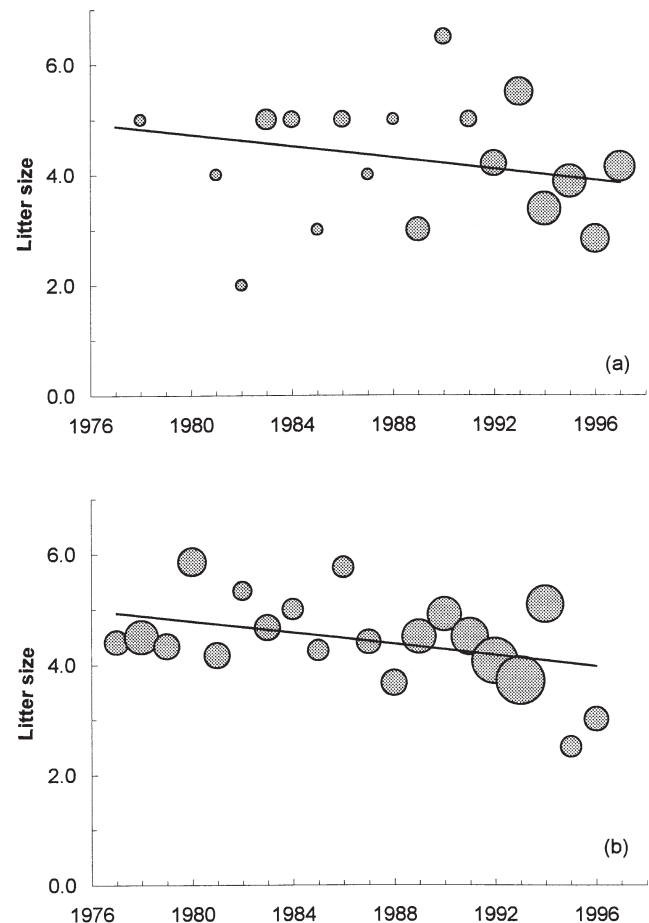
Discussion

Our examination of the effect of inbreeding upon viability and litter size in captive populations of Mexican and red wolves revealed no evidence for inbreeding depression. Year of birth was significantly associated with increasing viability; litter size showed an apparent decline over time.

Our conclusion that inbreeding did not affect viability in these two captive breeding programs is encouraging, as these programs seem to have less inbreeding depression than that for the Fenno-Scandic wolf (Laikre & Ryman 1991), although those wolves were much more inbred. In addition, the maximum number of lethal equivalents potentially present in both populations (Table 1) is approximately half the average number of lethal equivalents among mammals in captivity (Ralls et al. 1988). The upper bounds for $2B$, however, are not low enough to demonstrate a lack of inbreeding depression. But our analysis

warrants cautious optimism that inbreeding depression will not seriously lower the probability of wild populations of Mexican and red wolves surviving.

We recognize six potential limitations of our analysis. First, we examined only two of many aspects of fitness that inbreeding might affect. Our estimates of the number of lethal equivalents present in both populations can only be underestimates of the amount of deleterious genetic variation present in these populations. Second, we acknowledge that the upper bounds for 2*B* are not low enough to demonstrate a lack of inbreeding depression. Inbreeding may weakly affect viability. Interpretation of this conclusion must account for the fact that pedigrees managed to minimize inbreeding have exceptionally low power to demonstrate the absence of inbreeding depression (Kalinowski & Hedrick 1999). This limitation is caused by low amounts of inbreeding in the red wolf pedigree and by few levels of inbreeding in the Mexican wolf pedigree.

A third limitation of our analysis is the potential for unrecorded deaths to decrease estimates of inbreeding depression. Decreasing observation of individuals during and after birth, accompanied by cannibalism of inviable young, could explain both the trends toward increased viability and decreased litter size with time. Therefore, our estimates of the number of lethal equivalents and may be too low. Smaller litters, however, did not have a higher viability than larger litters. In addition, the data were fairly robust to this potential problem. Adding one nonviable birth to every litter of less then five increased our estimates of 2*B* from 0.00 and 0.00 to 0.42 and 0.00 for the Mexican and red wolves, respectively.

Another potential shortcoming of our analysis is nonindependence of viability among littermates. This concern led Lacy and Ballou (1998) and Lacy et al. (1996) to categorize litters of mice as viable or not depending on whether the majority of individuals survived. Such dependence would effectively lower the amount of information in the data, which would increase the size of confidence intervals for 2*B*. In particular, we might expect nonviability or sickness of one individual in the womb or den to have deleterious effects upon littermates. This would result in inflated estimates of lethal equivalents, which does not appear to have happened.

We also recognize that viability is potentially affected by variables not documented in studbooks. Although there are guidelines for raising captive Mexican and red wolves, husbandry conditions may have been unique for most litters in our analysis. There is no empirical or deductive method for incorporating these variables into models of fitness. We chose a logistic relationship between year of birth and viability because it is mathematically reasonable and could potentially summarize a trend in many unknown variables.

Finally, an absence of inbreeding depression in captivity does not ensure that inbreeding depression will be absent in more stressful environments. Evidence for increased inbreeding depression in stressful conditions has recently accumulated in both controlled (e.g., Miller 1994; Pray et al. 1994) and natural (e.g., Jimenez et al. 1994; Keller et al. 1994) environments.

Our conclusion that two wolf captive breeding programs had no evident inbreeding depression provides welcome news to wolf preservation efforts but little support to the hypothesis that natural inbreeding has purged deleterious genetic variation from wolf populations. Alleles at loci identical by descent in these breeding programs were descended from only three and nine founders for the Mexican and red wolf, respectively. Because founder effects can influence the effect of inbreeding (Lacy et al. 1996), this represents too small of a sample to make conclusions about an entire species.

Studbook analysis has provided conservation biology with important lessons (e.g., Ralls et al. 1988) and debates (e.g., Templeton & Read 1983; Ballou 1997; Lacy & Ballou 1998), but we must recognize and accept that breeding programs have different goals than experiments do. Studbook analysis is better suited to identify potential questions for research than for providing general principles for conservation biology. For example, Templeton and Read (1983) used studbook records to suggest that repeated generations of inbreeding within a captive breeding program could remove lethal genes from a population, and Lacy and Ballou (1998) experimentally examined this possibility with laboratory mice. Only rarely has inbreeding depression been examined experimentally in an endangered species (Sheffer et al. 1999). For conservationists, studbook analysis can provide a relatively cheap "best guess" answer for some questions. For Mexican and red wolves, our best guess is that there is low potential for inbreeding depression for the traits we examined, but we suggest our results be used conservatively and with healthy circumspection.

Literature Cited

Ballou, J. D. 1997. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. Journal of Heredity **88:** 169–178.

Cavalli-Sforza, L. L, and W. M. Bodmer. 1971. The genetics of human populations. Freeman, San Francisco.

Hedrick, P. W., P. S. Miller, E. Greffen, and R. Wayne. 1997. Genetic evaluation of the three captive Mexican wolf lineages. Zoo Biology **16:**47–69.

Jimenez, J. A., K. Hughes, G. Alaks, L. Graham, R. C. Lacy. 1994. An experimental study of inbreeding depression in a natural habitat. Science **266:**271–273.

Kalinowski, S. T., and P. W. Hedrick. 1998. An improved method for estimating inbreeding depression in pedigrees. Zoo Biology **17:** 481–497.

Kalinowski, S. T., and P. W. Hedrick. 1999. Detecting inbreeding depression is difficult in captive endangered species. Animal Conservation **2:**131–136.

Kärkkäinen, K., V. Koski, and O. Savolainen. 1996. Geographical variation in the inbreeding depression of Scots pine. Evolution **50:**111–119.

Keller, L. F., P. Arcese, J. N. M. Smith, W. M. Hochachka, and S. C. Stearns. 1994. Selection against inbred Song Sparrows during a natural population bottleneck. Nature **372:**356–357.

Lacy, R. C. 1997. Errata. Evolution **51:**1025.

Lacy, R. C., and J. D. Ballou. 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. Evolution **52:**900–909.

Lacy, R. C., G. Alaks, and A. Walsh. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus.* Evolution **50:** 2187–2200.

Laikre, L., and N. Ryman. 1991. Inbreeding depression in a captive wolf (*Canis lupus*) population. Conservation Biology **5:**33–40.

Laikre, L., N. Ryman, and E. A. Thompson. 1993. Hereditary blindness in a captive wolf population: frequency reduction of a deleterious allele in relation to gene conservation. Conservation Biology **7:** 592–601.

Miller, P. S. 1994. Is inbreeding depression more severe in a stressful environment? Zoo Biology **13:**195–208.

Morton, N. E., J. F. Crow, and J. H. Muller. 1956. An estimate of the mutational damage in man from data on consanguineous marriages. Proceedings of the National Academy of Sciences of the United States of America **42:**855–863.

Nowak, R. M, and N. E. Federoff. 1998. Validity of the red wolf: response to Roy et al. Conservation Biology **12:**722–725.

Pray, L. A., and C. J. Goodnight. 1995. Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum.* Evolution **49:**176–188.

Pray, L. A., J. M. Schwartz, G. J. Goodnight, and L. Stevens. 1994. Environmental dependency of inbreeding depression: implications for conservation biology. Conservation Biology **8:**562–568.

Ralls, K. J., J. D. Ballou, and A. R. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology **2:**185–193.

Sheffer, R. J., P. W. Hedrick, and A. Velasco. 1999. Testing for inbreeding and outbreeding depression in the endangered Gila topminnow. Animal Conservation **2:**121–129.

Siminski, D. P. 1998. International studbook for the Mexican gray wolf. Arizona-Sonora Desert Museum, Tucson.

Sokal, R., and F. J. Rohlf. 1995. Biometry. 3rd edition. W. H. Freeman, San Francisco.

Templeton, A. R., and B. Read. 1983. The elimination of inbreeding depression in a captive herd of Speke's gazelle. Pages 241–261 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. Genetics and conservation. Benjamin/Cummings, Menlo Park, California.

Waddell, W. 1997. Studbook for the red wolf. Point Defiance Zoo and Aquarium, Tacoma, Washington.

Wayne, R. K., D. A. Gilbert, A. Eisenhawer, N. Lehman, K. Hansen, D. Girman, R. O. Peterson, L. D. Mech, P. J. P. Gogan, U. S. Seal, and R. J. Krumenaker. 1991. Conservation genetics of the endangered Isle Royale gray wolf. Conservation Biology **5:**41–51.

Wayne, R. K., M. S. Roy, and J. L. Gittleman. 1998. Origin of the red wolf: response to Nowak and Federoff and Gardner. Conservation Biology **12:**726–729.