IMPACTS OF INBREEDING IN NATURAL AND CAPTIVE POPULATIONS OF VERTEBRATES: IMPLICATIONS FOR CONSERVATION

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Many wildlife populations that were once large, widespread, and diverse have been reduced to small, isolated populations in a few remaining natural areas, nature preserves, and zoological parks. Once a population becomes very small and isolated from potential sources of immigrants, random demographic and genetic processes can lead the population rapidly toward extinction. Demographic problems faced by small, fragmented populations include difficulty in finding mates, random skewing of the sex ratio, and simply the misfortune of all animals dying or failing to breed in a given year due to chance, a disease outbreak, a temporary scarcity of food or an abundance of predators, or local weather conditions. These and other random demographic and environmental events can cause a small population to decline still further. Even if the causes of such a decline are temporary, the resulting very small population may be forced to inbreed. It has long been observed that inbreeding causes greater mortality and reduced fecundity in many species, the phenomenon called "inbreeding depression" [1–3]. Inbreeding and the loss of genetic diversity may lower fitness and reduce the potential for the population to adapt. This can lead to further decline, making the demographic problems worse, in turn making the genetic problems worse—a feedback that drives small populations ever faster toward extinction. This process has been termed the "extinction vortex" [4], and the size below which a population is likely to get drawn into the extinction vortex is one useful definition [e.g., 5] of the Minimum Viable Population size (or MVP) [6].

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Conservation of remnant wildlife populations requires knowledge of the minimum population size below which the combined effects of random genetic changes and demographic variation would likely result in extinction [4–11]. Estimating the MVP for a population will require considerable knowledge of the effects of inbreeding on endangered populations [12]. In particular, we need to know: (1) whether the inbreeding depression that is so prevalent among domesticated animal stocks is equally characteristic of most wild populations; (2) the severity of inbreeding depression (the function relating fitness traits to past and present inbreeding) in natural populations; (3) whether inbreeding depression is consistent and predictable; and (4) whether a depression in fitness traits can be avoided or reversed by combining inbreeding with selection to purge a population of the genes that cause inbreeding depression.

Causes of Inbreeding Depression

The common explanation for the hazards of inbreeding (inbreeding depression) rests on the presence of deleterious recessive genes, a "genetic load," in virtually all diploid organisms. Natural selection will reduce the frequencies of deleterious alleles in a population (or keep newly arisen harmful mutations from becoming common), but it is very inefficient at eliminating wholly a recessive allele because such alleles are almost always shielded from selection in heterozygotes. One consequence of inbreeding is that it makes it much more likely that an individual is homozygous for a rare gene because it is more likely that two related parents simultaneously possess a rare allele and transmit it to their inbred offspring than that two unrelated individuals independently transmit the same rare allele to a non-inbred offspring. Thus, inbreeding seems to reduce fitness because it reveals harmful genes in homozygotes.

If this explanation of inbreeding is correct, then we should be able to predict which populations would be most susceptible to inbreeding depression [12]. When inbreeding occurs, deleterious genes will be expressed in homozygotes and cause the death of individuals bearing them, but those genes are thereby eliminated from the population. Thus, populations with histories of inbreeding should now be largely purged of their genetic load of recessive deleterious genes. On the other hand, populations that have long been large and diverse may harbor many, individually rare, deleterious, recessive genes.

Thus, the black-footed ferret (*Mustela nigripes*), which probably existed in numbers no greater than 50 breeding adults during the past 50 years [13] and which was extinct in the wild [14] until reintroduction of captive-born ferrets began in 1991, may harbor few deleterious recessive

alleles and may be unaffected by the inbreeding that will necessarily occur as the small remnant population is propagated in captivity and restored to natural habitats. Similarly, endangered species that are endemic to small islands may suffer little depression in fitness when inbred. Species that declined to very low numbers only within the past generation or two, such as the California condor (*Gymnogyps californianus*) and black rhinoceros (*Diceros bicornis*), might still have large genetic loads and may be unable to survive even moderate inbreeding. The greater one-horned rhinoceros (*Rhinoceros unicornis*), which declined from thousands of animals prior to 1950 to fewer than 100 animals during the 1960s, retains high levels of genetic variation [15] and presumably still has a genetic load typical of nonendangered mammals.

An alternative explanation of inbreeding depression is that heterozygotes may be more adaptive than homozygotes for many or even most genetic loci. Lerner [16] proposed that such overdominance, or heterosis, may be a general phenomenon, with heterozygotes showing greater developmental stability and greater ability to tolerate environmental fluctuations and stresses. If such general heterosis is the primary cause of inbreeding depression, then we would expect fitness to decline monotonically as a population becomes inbred and loses heterozygosity. Little adaptation to inbreeding would occur because selection favoring heterozygotes maintains balanced polymorphisms and therefore retains, rather than removes, the genetic load. Only indirect selection on modifier loci to reduce the heterozygote advantage, perhaps by providing alternative biochemical pathways that make the heterotic locus superfluous, would effectively remove a genetic load resulting from heterosis. If general heterosis is the primary cause of inbreeding depression, then populations that have undergone prolonged bottlenecks might already have declined in fitness and might be the most vulnerable to further losses of genetic variation.

Clearly, whether inbreeding depression results primarily from deleterious recessive alleles or general heterosis is of considerable importance to conservation. The design of wildlife preserves, breeding programs, and recovery plans all require estimation of MVPs, which in turn depends upon estimates of the sensitivity of populations to inbreeding depression. We need to know which populations could survive the inbreeding that accompanies a severe bottleneck, and which would suffer irreversible decline in fitness if the population declined to numbers that forced matings between close relatives [12].

Inbreeding in Domesticated and Laboratory Animals

For hundreds of years, animal breeders have been aware of the problems associated with close inbreeding. Darwin [1] documented exten-

sively inbreeding depression in domesticated stocks, and Wright [2], Falconer [3], and others have reviewed data that have accumulated since. A very large body of data demonstrates that inbreeding depresses virtually every component of fitness that has been measured in each species of domesticated livestock. Fertility, birth weight, growth rate, survival, disease resistance, productivity (of meat, milk, eggs, wool, etc.) are all depressed when farm animals are inbred. Attempts to completely inbreed cattle, sheep, and other species have always ended in extinction of the stock, suggesting that it may not be possible to rid these populations entirely of their genetic loads. The failure to produce a viable, fully inbred stock of any domesticated livestock species is unexpected if inbreeding depression in livestock is due to fully recessive, deleterious genes. The lack of inbred strains of livestock suggests instead that heterozygote vigor at some loci or throughout much of the genome is essential to survival and/or reproduction.

Studies on inbreeding in laboratory rodents have yielded equivocal evidence on the nature of inbreeding depression. Although many inbred lines of mice and rats have been produced for biomedical research, few data were kept on the breeding records during the course of inbreeding [17]. What we know about the effects of inbreeding on those lines comes primarily from subsequent crosses between stocks to produce outbred mice [3]. More recently, researchers have inbred newly wild-caught house mice and documented the inbreeding depression that resulted [18, 19]. Many sublines went extinct during the production of the viable inbred lines, as would be expected if selection were removing deleterious alleles from the population during the course of inbreeding. None of the inbred lines of rodents, however, are as fit as are outbred stocks produced by crossing inbred lines or obtained from the wild. Thus, either some deleterious recessive alleles were fixed within each of the inbred lines, or fitness at heterotic loci was sacrificed during the production of the inbred lines. The data do not distinguish between causes of inbreeding depression.

Inbreeding in Captive Populations of Wild Animal Species

Domesticated animals and most laboratory stocks result from hundreds to thousands of years of intense artificial selection, and it is not clear that they are good models for natural populations. Zoological parks collectively breed thousands of vertebrate species, and either because of a lack of more suitable animals to pair or because of a lack of concern about the effects of inbreeding, zoos often inbreed their stocks. The following summary of the impacts of inbreeding on zoo populations is developed more fully by Lacy, Petric, and Warneke [20].

Scattered through the scientific literature are case studies of inbreed-

ing in species propagated by zoos and private breeders. For every species studied in detail, investigators report that inbreeding depresses at least some aspect of fitness in some populations, with infant mortality being the most commonly measured fitness component. However, different species and even different captive stocks of the same species often respond to inbreeding in divergent ways. For example, Shoemaker [21] found that inbreeding increased juvenile mortality in Persian leopards, but he reported no measurable effect on juvenile mortality in Chinese leopards or Amur leopards. Daniell and Murray [22] reported inbreeding to depress nestling survival in one colony of budgerigars, but not in another. Slatis [23] found higher mortality in inbred European bison, but only among the descendants of a male that was from a different subspecies than the rest of the remnant world population. Either this male harbored recessive lethal genes not found in the other bison, or the reported "inbreeding depression" was actually hybrid breakdown that occurred after hybridization between the subspecies.

Kathy Ralls and Jon Ballou of the National Zoological Park have compiled data on the effects of inbreeding on zoo populations (see [24] for a review). Examining 45 species of mammals bred in zoos, they showed that juvenile mortality was usually higher in inbred animals, but they did not quantify the severity of inbreeding depression in these zoo stocks. More recently, Ralls, Ballou, and Templeton [25] presented a more detailed study of the severity of inbreeding depression in 40 populations of mammals. They calculated the number of "lethal equivalents" per diploid individual, measured by regressing the logarithm of juvenile survival against the inbreeding coefficient. Lethal equivalents quantify the genetic load by estimating the number of recessive lethal alleles per individual if the observed inbreeding depression were due solely to lethal, fully recessive genes [26]. Ralls et al. [25] reported the effect of inbreeding depression on juvenile mortality to range from -1.36 lethal equivalents in maned wolves (*Chrysocyon brachyurus*) to 30.32 in Wied's red-nosed rat (*Wiedomys pyrrhorhinos*). (Although the negative value measured in maned wolves was not significantly less than zero, negative lethal equivalents would indicate greater survival in inbred litters than in noninbred offspring). The survey detected no clear trends among the mammalian orders. Nor did it demonstrate a difference between populations started from wild-caught animals and those started from stocks that had already been in captivity for one or more generations, which may have already had some of the genetic load removed by selection during previous episodes of inbreeding.

Recently, colleagues at the Chicago Zoological Park and I compiled and analyzed records for species of Artiodactyla with long-term breeding histories at the zoo [20]. When appropriate to the social system of the species, the Chicago Zoological Park maintains its hoofstock in herds.

Because of limited availability of unrelated stock, many herds were founded with only a few individuals, and often decades pass before unrelated animals are added to the herds. Thus, each population becomes progressively inbred through several generations, until a new breeding animal (usually a male) is introduced to outcross the herd. Like Ralls and Ballou, we assessed the impact of inbreeding on just one aspect of fitness, infant mortality prior to 30 days.

For two species, the banteng (*Bos javanicus*), a wild cattle species from southeast Asia, and the wisent or European bison (*Bison bonasus*), complete pedigree records allowed calculation of inbreeding coefficients for 33 and 63 descendant animals, respectively. In both species, infant mortality increased as the populations became inbred, although the trend was not statistically significant for the wisent. We estimated the number of lethal equivalents to be 2.33 for the banteng and 1.05 for the wisent. Although these estimates are based on herds established from very few animals (two and three, respectively), it is not surprising that the estimated genetic load of the wisent, a species which has been through a severe bottleneck and which was inbred prior to importation to the U.S.A. [23], is less than half the load of lethal equivalents estimated for the banteng, a species that has a much broader extant range and is not known to have been forced to inbreed during bottlenecks prior to its captive history. It cannot be determined, however, whether the genetic load of the wisent was reduced during the historical bottleneck, or whether its recovery from the bottleneck was facilitated by a smaller genetic load that existed even prior to the bottleneck.

For several other species of bovids bred at the Chicago Zoological Park, incompleteness of pedigrees prevented calculation of inbreeding coefficients. A large herd of Siberian ibex (*Capra ibex sibirica*) were begun from a single male and four females that were brought to the zoo 25 years ago. The herd has been maintained as a closed breeding stock since that time, and it could only have become progressively inbred (although at an unknown rate) during five to ten generations of captive breeding at the zoo. Infant mortality of the ibex was found to have increased significantly through the years, with a sudden increase in mortality coinciding with the replacement of a first-generation male by third and later generation (and therefore highly inbred) males as the dominant breeders in the herd.

For two large antelope species, sitatunga (*Tragelaphus spekei*) and addax (*Addax nasomaculatus*), herds were begun with a single pair more than 50 years ago, but breeding males were supplemented or replaced by unrelated animals every few generations. Thus, there was an alternation between periods of inbreeding and outbreeding within the herd. For both of these species, infant mortality was low in the first few years after each introduction of a new, unrelated breeding male, followed by

significant increases in infant mortality after the herd had been kept closed long enough for the breeding males to be mating with daughters and granddaughters. Because of hunting, addax are very rare and possibly almost extinct in the wild. The few remaining addax in Niger (no addax have been seen outside of Niger in recent years) likely live in family groups even smaller than the herd of 10 to 20 animals kept at Brookfield, and may now be even more inbred than are the zoo animals.

Inbreeding Depression in *Callimico*

Ideally, the effects of inbreeding on captive wildlife should be measured on a single captive population in which it can be documented that inbred and non-inbred animals were given the same care. Except for some ungulate species, like those cases described above, few species are kept in sufficient numbers in any one zoo to permit statistical analysis of the effect of inbreeding on mortality or other measures of fitness. For many species, however, international studbooks document the breeding records from many zoos. If incidences of inbreeding are widely distributed across a number of zoos, then it may be reasonable to assume that differences in mortality of inbred and noninbred animals are not solely due to differences in management among zoos.

One species with a particularly complete and probably accurate studbook is the Goeldi's monkey (*Callimico goeldii*). This monotypic genus is evolutionarily between the Callithricidae and the Cebidae, and often is accorded its own family, the Callimiconidae [27]. Pedigrees of most captive-born *Callimico* can be traced back to wild-caught ancestors from a few importations; paternity is rarely uncertain (because they are maintained as monogamous pairs), and most aborted fetuses, stillbirths, and neonatal deaths have been recorded. The international studbook [28, and unpublished updates] for captive *Callimico* now contains more than 1,000 animals. After eliminating all animals for which there is question as to one or more of the captive ancestors, complete pedigree data are available on 111 inbred and 679 non-inbred captive *Callimico* (including stillbirths and abortions). Working with the studbook keeper, I examined the effect of inbreeding on infant mortality before 30 days of age [20].

Callimico showed the most severe inbreeding depression reported in any vertebrate species for which large sample sizes are available for analysis. Each 10% increase in inbreeding resulted in a 33% decrease in survival. The genetic load was estimated as 7.90 lethal equivalents per diploid genome. Separate analysis of abortions, stillbirths, and infant deaths revealed twofold greater mortality among inbred than non-inbred progeny during each developmental stage. The effects of inbreeding on female infants were found to be much more severe than

on male infants (10.9 vs. 3.2 lethal equivalents), with female progeny from first-cousin matings showing less than half the viability of non-inbred offspring.

Zoo animals are sheltered from many of the causes of mortality that afflict natural populations (e.g., predation, food stress, extremes of weather, epidemic disease), and reduced nongenetic mortality relative to natural populations could lead to greater statistical sensitivity in detecting inbreeding depression. Yet, the benevolent captive environment, while removing many nongenetic causes of mortality, might also minimize the impact of the genotype on survival. Many deleterious alleles would affect fitness only during times of stress, and genetically handicapped animals often live much longer in captivity than they could in the wild. For example, weak infants and those rejected by their mothers are often hand-raised by zoos. Thus, while zoo breeding records provide an opportunity to gather data on much larger pedigrees than could be obtained from studies of wild populations, it should be recognized that they probably provide minimum estimates of the effects that inbreeding would have on populations subjected to the stresses of a more natural environment.

In summary of the cases presented thus far: Extensive data are available to document the deleterious effects of inbreeding on the survival and reproduction of domesticated animals. Studies utilizing laboratory strains of rodents demonstrate that viable, fully inbred strains can be developed, but they have reduced fitness relative to more outbred stocks. Data from zoos demonstrate that the phenomenon of inbreeding depression is widespread among nondomesticated species as well, causing increased infant mortality even in the nurturing environment of zoos. Data on zoo populations suggest also that the severity of inbreeding depression can vary widely among species, populations, and even sexes, but no trends have been identified that would allow prediction, *a priori*, of the quantitative impact of inbreeding on populations that have not yet been studied.

Inbreeding in Insular and Central Populations

To examine the causes of inbreeding depression and to develop quantifiable predictors of the severity of inbreeding depression in populations, Bruce Brewer, I, and colleagues collected mice of the genus *Peromyscus* from island populations and from otherwise isolated populations that had undergone severe declines in past decades, and also from large and stable mainland populations. Brewer established laboratory colonies from eight of the populations and began an extensive study of the effects of inbreeding on each population [29]. The results of that study, summarized briefly below, are presented in detail by Brewer et al. [30].

Several predictions were made regarding the effects of inbreeding on the mouse populations. First, because of the small size and long isolation of the insular and peripheral populations, they were expected to have less genetic variability, when assayed by gel electrophoresis of protein (allozyme) variation, than would the larger, contiguous, mainland populations. Second, the more isolated populations were expected to have smaller genetic loads and therefore to show less depression of fitness traits when forced to inbreed in the laboratory. If these predictions could be confirmed (not only with these mouse populations, but also with a number of species), then conservation programs may be able to use knowledge of the size and history of natural populations to predict the severity of inbreeding depression. Moreover, even if historical data on population size and isolation were lacking, assays of extant genetic variation would be indicative of the size of the genetic load.

Laboratory populations were established from six populations of five subspecies of *Peromyscus polionotus*, and two populations of distinct subspecies of *P. leucopus*. *P. p. leucocephalus*, a subspecies endemic to Santa Rosa Island along the Gulf coast of northwestern Florida, has been isolated from the mainland for several thousand years and is well differentiated morphologically, behaviorally, and genetically from inland subspecies. Hurricanes periodically decimate the coastal and island populations, and three other subspecies that are endemic to nearby islands and coastal areas are now listed as Endangered by the U.S. Fish and Wildlife Service [31]. *P. p. phasma* formerly inhabited the Atlantic coastal area of northeastern Florida, but has been restricted just to sections of Anastasia Island for the last few decades. This subspecies is now listed as Endangered. *P. p. niveiventris* once inhabited much of the eastern coast of Florida, from Miami to Daytona, but is now restricted to the federally protected habitat on Cape Canaveral. *P. p. rhoadsi* inhabits an inland region of south-central Florida, at the southern end of the present range of the species. Although still widespread, most of the habitat on which it relies (deep, well-drained, sandy soil) has been converted to citrus groves, and mouse densities appear to have declined over the past few decades. Mice from two populations of the subspecies *P. p. subgriseus* were collected from north-central Florida, in areas of extensive habitat. This subspecies is still widespread and abundant. Two subspecies of *P. leucopus*, *P. l. noveboracensis* from the deciduous forests of the northeastern U.S. and *P. l. tornillo* from Texas, were collected. Both populations inhabit expansive areas of habitat.

Genetic variability within each population was assayed by gel electrophoresis of 30 enzymes in blood, liver, kidney, and muscle samples. The wild-caught mice that were used to initiate lab stocks were analyzed and were found to accord with our predictions: Those populations that are large and abundant showed relatively high levels of genetic variability

(ranging from 8% to 13% heterozygosity), while the island populations had only one-half to one-third as much allozyme variation. Presumably, the island populations have lost variability while inbreeding during previous population crashes.

Each generation in the lab, some mice were paired with relatives to produce inbred offspring. Others were paired with nonrelated mice (of the same population) to produce outbred control litters. To assess the effects of inbreeding, we determined the relationship between several fitness components and the level of inbreeding of litters. We monitored the number of offspring born per litter, the survival of young until weaning at 20 days of age, and the mass of young at weaning. The severity of inbreeding depression was measured by the slope of each of these variables regressed against the inbreeding coefficient of the litters, after the effects of parity of the dam, age of the dam, inbreeding of the dam, and inbreeding of the sire were factored out. Logarithmic transformations were applied to each fitness measure prior to the regression analysis, and this measure of inbreeding depression for juvenile survival estimates the number of lethal equivalents per haploid genome [25, 26].

Unlike the clear trend relating genetic variation to the degree of isolation of populations, the responses of the populations to inbreeding did not follow predictions. First, different components of fitness were affected by inbreeding in the different populations. Some populations produced fewer offspring per litter when inbred, others had reduced growth of inbred litters, and yet a different set of populations suffered greater mortality. Thus, independent genetic factors must be controlling the response of these components of fitness to inbreeding. The ecological, demographic, selective, or historical determinants of the genetic loads of these populations must have acted differently on the various components of fitness because the relative rankings of inbreeding depression depended critically on which components of fitness were examined. Moreover, for none of the fitness measures was there a significant association between the measures of extant genetic variation and the severity of inbreeding depression. Thus, the island populations, which are depauperate in genetic variability and probably have had a history of inbreeding during past population declines, had no lower genetic loads than did mainland populations with more allozyme diversity. Moreover, within each population, inbred offspring of inbred parents fared no better than did inbred offspring of outbred parents, as would have been expected if the genetic load consisted primarily of recessive deleterious alleles that would be removed by selection during repeated generations of inbreeding.

Overall, the effects of inbreeding, both which aspects of life history are affected and the severity of the inbreeding depression, were not

predictable from knowledge of the genetics of the natural populations. This suggests that the effects of inbreeding on any given trait are controlled by a small number of genes and that the presence or absence of those genes in a population is more or less a chance phenomenon. Some populations may be unlucky and harbor a large genetic load, either due to recessive deleterious alleles or due to heterozygote vigor. Other populations may not have genes that would be problematic under inbreeding, but the difference is probably determined by historical accident, not easily identified aspects of population history. The establishment of our lab stocks was similar to the establishment of many reintroduced populations, zoo stocks, and natural recolonizations. In each case, a small number of founders (perhaps 5 to 10) from one population become the nucleus for a new population. Our findings suggest that we are not yet able to predict the severity of inbreeding depression likely to impact recovery programs for endangered species, nor even what traits are likely to be most affected by inbreeding. We must develop and confirm theories adequate to predict accurately the diversity of responses to inbreeding by wild populations. Until we do so, we will have to determine empirically the severity of inbreeding depression and the likely role of genetic processes in extinction or efforts at recovery for each endangered population.

Inbreeding depression is an almost universal phenomenon, and it can impact wild animal species as well as domesticated stocks, but its severity is not yet predictable. There is not yet convincing evidence that selection will commonly adapt populations to inbreeding by removing the genetic load. Yet all of the data that I have presented above have been based on nonnatural populations, either in laboratories or in zoological parks. Thus, an important question remains: Does inbreeding depression contribute to the decline and extinction of wild populations?

The Effect of Inbreeding on Natural Populations

It has been argued by many, and at times even by me, that while inbreeding depression is a serious concern for captive breeding programs, in which the goal would be to maintain a small, healthy population in captivity for many generations, habitat loss and demographic problems, not inbreeding, cause the demise of populations in the wild. Captive populations can be protected from so many of the risks that endanger wild populations that they can be propagated for many generations at numbers so low as to lead to severe inbreeding. Wild populations are unstable demographically when they decrease to numbers that would force inbreeding. Very small wild populations would become extinct before many generations pass, and therefore before there is an opportunity for inbreeding depression to exacerbate the decline.

While no natural population is known to have gone extinct because of inbreeding depression, there is increasing evidence that inbreeding depression is contributing to the decline, or impeding recovery, of some wild populations. Certainly, some wildlife populations are now so small that inbreeding is common or even inevitable. For example, the Florida panther (*Felis concolor coryi*) is the only remaining subspecies of puma or mountain lion in the eastern U.S.A. Only about thirty adults remain, and those are divided into two isolated subpopulations [5]. Of the five litters of kittens known to have been born in 1989, one was the product of a father-daughter mating, another was from a mother-son mating, and the other three were of uncertain paternity (two were likely sired by the male that mated to his daughter, and it is likely that he is also the sire of at least one of those two dams). While inbreeding depression (as opposed to just inbreeding) within this tiny population cannot be proven without extensive studies, it is notable that most of the male Florida panthers are cryptorchid (having just one descended testicle), some have congenital heart defects, and several of the traits considered to be diagnostic of the subspecies (a whorl of fur on the back and a kink in one of the tail vertebrae) are probably nonadaptive genetic abnormalities that have become fixed by chance in the small population. The sensitivity of black-footed ferrets, bighorn sheep, and other endangered species to viral diseases and parasites may also be related to a lack of genetic variability resulting from years of inbreeding in remnant populations [32, 33].

There is a great need for studies of the effects of inbreeding on remnant wild populations, but it is difficult to demonstrate the effects of inbreeding in the wild. For example, in the case of the Florida panther, although it is likely that cryptorchism and other developmental anomalies result from the prevalence or fixation of deleterious alleles in that small population, no studies have been conducted to show definitively that these traits have a genetic basis in that population. Lacking field data on inbreeding effects with which to help design a recovery program for the population, I investigated the *likely* impact of inbreeding on the Florida panther population as part of simulation modeling done in conjunction with a Population Viability Analysis workshop convened by the Captive Breeding Specialist Group of the Species Survival Commission (IUCN—The World Conservation Union) and state and federal agencies [5]. A computer program was used to simulate demographic, environmental, and genetic stochasticity. Birth and death rates and the present population structure were estimated from field data and entered into the program. Other examples of the use of this program are given by Lacy et al. [10] and Lacy and Clark [11].

To examine the possible effects of inbreeding on the persistence of the remnant population of Florida panthers, I simulated the population

10,000 times, monitoring changing population sizes and times to extinction. The simulations were repeated under several different scenarios regarding the genetic load of the population. Even in the absence of inbreeding depression, the simulated populations went extinct, on average, in about the year 2030 with a distribution of extinction dates ranging from 2000 to 2100. Three levels of inbreeding depression were modeled: genetic loads of 1.0, 1.7, and 3.4 lethal equivalents. The last case corresponds to the median level of inbreeding depression observed by Ralls, Ballou, and Templeton in their study of 40 populations of mammals in zoos [25]. We presently have no data on the genetic load in Florida panthers, or in any wild felid population. With increasing severity of inbreeding depression, the mean time to extinction of the simulated populations decreased only slightly: the Florida panther population will probably go extinct within 30 to 50 years whether or not fitness declines as the population becomes inbred. The incorporation of inbreeding in the models, however, did remove the right-hand tail of the distribution of times to extinction. If inbreeding affects Florida panthers as much as it does the typical mammal, there is virtually no probability that the population will survive more than 40 years. Inbreeding depression removes the possibility that the panthers will get lucky and happen to survive random demographic and environmental fluctuations into the second half of the next century. This occurs because inbreeding depression reduces fitness as soon as the population becomes very small, rapidly driving it to extinction (the extinction vortex). In the absence of inbreeding depression, some of the simulated populations recover from population crashes and survive for decades beyond an initial decline.

Further insight is gained by examination of the decline in genetic variation in the simulated populations. Selection for heterozygotes, which occurs under the mode of inbreeding depression (general heterosis) modeled in the simulation, was ineffective at retaining genetic variability. At very small population sizes, genetic drift overwhelms selection, and deleterious genes are as likely to be fixed as are advantageous alleles [34]. Simulated populations became extinct after losing 40% to 70% of the initial genetic variation. These simulations are consistent with much experimental work, which suggests that it is very difficult to retain a vertebrate population even for a few generations that has inbreeding coefficients as high as $F = .50$.

With respect to management of the Florida panther, these results suggest that if we wait until the population is very small (on the order of 10 animals) before very aggressive management actions are taken to reverse the decline, it will likely be too late. Similarly, and tragically, it is entirely possible that the California condor, the black-footed ferret, and other species receiving much attention and millions of dollars in recovery efforts may have been so damaged genetically prior to the

recent management efforts that recovery of healthy populations in the wild may be impossible.

Hybrid Vigor: Indirect Evidence of Inbreeding Depression

Recently, my colleagues and I at the Chicago Zoological Park have been examining the role that genetic variation may play in maintaining the health of natural populations, not by looking at the effects of forced inbreeding, but rather by examining the response of populations to increases in genetic variation resulting from hybridization among divergent populations of the same species. We have made 20 to 30 crosses between each pair of the six populations of *Peromyscus polionotus* used in the inbreeding experiments [29, 30] described above. (Only noninbred control stocks were used for this study, not the artificially inbred mice produced during the inbreeding experiments.) These populations include two local samples of one subspecies, several closely related subspecies, and also some subspecies (e.g., the island forms) that are sufficiently distinctive that some biologists have suggested that they merit consideration as full species. Hybrid progeny produced by these crosses were subsequently bred to produce second-generation (F2) between-population hybrids.

As in the inbreeding experiments, we monitored the number of offspring per litter, the survival of young to weaning, and the mass of offspring at weaning. In addition, we controlled the length of time that each pair was left together (63 days) and examined the proportion of pairs producing litters during that time. We expected that the matings between closely related subspecies would produce litters as large and healthy as those of the within-population control matings, but we thought that crosses between strongly divergent subspecies (e.g., those involving the Santa Rosa Island endemic population) might show reduced fitness due to incompatibility of hybrid genotypes. Often, first-generation hybrids between divergent populations or even biological species display hybrid vigor, while hybrid breakdown occurs when these hybrids are mated to produce second-generation hybrids [35, 36].

Our preliminary results (not yet published) have not supported our predictions. For every fitness component measured, there was strong hybrid vigor. This effect was even stronger in the F2 generation, perhaps due to good parental care given by F1 hybrid dams. Moreover, the isolated populations that are the most divergent genetically, and that have the least genetic variation, were the ones that showed the strongest hybrid vigor. Crosses between similar, and highly variable, mainland populations showed little or no hybrid vigor. This suggests that the isolated populations are already experiencing depression of fitness due

to the loss of genetic variation; inbreeding depression is suppressing fitness in the existing natural populations.

One of the two island populations included in this study is declining steadily and is now listed as Endangered, and the other is being reviewed for possible listing as Threatened. Of five other isolated subspecies of beach mice, a peninsular population (also used in this study) is listed as Threatened, an island population and two coastal populations are Endangered, and one coastal form is already extinct. Conversion of the habitat into residential areas and public beaches has limited the mouse populations to very narrow strips of coastal dunes, often in areas that are highly susceptible to damage by storms (and therefore unsuitable for residential development by humans). Hurricanes and house cats have decimated these remnant, local populations of beach mice. It now seems that losses of genetic variation have reduced the viability and reproduction of the more endangered populations, and that recovery of the populations may not be possible even if they are afforded protection from further habitat damage.

Conclusion

Inbreeding depression is likely to be one of the causal factors in the extinction of wildlife populations. Inbreeding will only become important after other factors have already reduced the populations to critically low numbers, but many wildlife populations are now down to so few breeding animals that genetic problems are likely to compound the more obvious causes of decline. While some theorists state that genetic considerations are unimportant in the conservation of wildlife populations because inbreeding depression only begins when the size of isolated populations are below 50, many wildlife agencies are failing to take aggressive action to reverse population declines until numbers drop as low as 10 or 20. Inbreeding effects will make the recovery of some endangered species even more difficult than has been presumed heretofore.

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