INBREEDING DEPRESSION IN CONSERVATION BIOLOGY

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■ **Abstract**   Inbreeding depression is of major concern in the management and conservation of endangered species. Inbreeding appears universally to reduce fitness, but its magnitude and specific effects are highly variable because they depend on the genetic constitution of the species or populations and on how these genotypes interact with the environment. Recent natural experiments are consistent with greater inbreeding depression in more stressful environments. In small populations of randomly mating individuals, such as are characteristic of many endangered species, all individuals may suffer from inbreeding depression because of the cumulative effects of genetic drift that decrease the fitness of all individuals in the population. In three recent cases, introductions into populations with low fitness appeared to restore fitness to levels similar to those before the effects of genetic drift. Inbreeding depression may potentially be reduced, or purged, by breeding related individuals. However, the Speke's gazelle example, often cited as a demonstration of reduction of inbreeding depression, appears to be the result of a temporal change in fitness in inbred individuals and not a reduction in inbreeding depression.

Down, July 17, 1870

My Dear Lubbock,
...In England and many parts of Europe the marriages of cousins are objected to from their supposed injurious consequences: but this belief rests on no direct evidence. It is therefore manifestly desirable that the belief should be either proved false, or should be confirmed, so that in this latter case the marriages of cousins might be discouraged...

   It is moreover, much to be wished that the truth of the often repeated assertion that consanguineous marriages lead to deafness and dumbness,

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blindness, &c, should be ascertained: and all such assertions could be easily tested by the returns from a single census.

> Believe me,
> Yours very sincerely,
> Charles Darwin

INTRODUCTION

The detrimental effects of close inbreeding on traits related to fitness have long been documented in humans and organisms commonly bred by humans (20, 21). However, the impact of inbreeding upon endangered species was not considered until 1979 (82), when it was documented that inbreeding lowered the juvenile survival in 41 of 44 populations of captive ungulates. In a follow-up survey, 36 of 40 captive populations exhibited decreased juvenile viability in inbred animals, although there was extensive variation among the estimates, and some populations had statistically nonsignificant inbreeding depression (81). Supporting the ubiquity of inbreeding depression, Lacy (51, p. 331) stated that he was "unable to find statistically defensible evidence showing that any mammalian species is unaffected by inbreeding. Moreover, endangered species seem no less impacted by inbreeding, on average, than are common taxa." Even the cheetah, argued to be already so inbred and low in fitness that further inbreeding would have no effect (75), has been shown to have inbreeding depression (32, 102). General recognition of the potential negative effect of inbreeding on fitness has made inbreeding depression a concern in small-population conservation and inbreeding avoidance a priority in captive breeding of endangered species. Therefore, we concentrate here on reviewing literature concerned with the relationship of inbreeding depression to conservation, primarily in endangered animal species.

Thorough reviews have covered various aspects of inbreeding depression such as the evolution of inbreeding (14, 97) and the purging of inbreeding depression in plant populations (12). Further, the effect of inbreeding on different categories of traits has also been considered as, for example, the magnitude and timing of inbreeding depression in plants (40), inbreeding depression of different fitness components or genetic abnormalities (56), and comparison of inbreeding depression for life-history and morphological traits (22). In addition, some recent reviews have discussed the amount of genetic variation for fitness components and the factors affecting the mutation-selection balance for these traits (15, 64). The genetic basis of inbreeding depression has been considered and debated for many years (14a). Recent experimental work is having some success in characterizing the genetic variation underlying inbreeding depression (23, 42, 83, 103, 104). The ability to map genes (QTLs) (68) affecting fitness-related traits portends imminent knowledge of the detailed architecture of genes affecting inbreeding depression, i.e. the number

and location of the genes, the distribution of their effects and their dominance, and the interaction (epistasis) of different genes. There has also been discussion of other theoretical concepts relevant to our review (13, 30, 39, 72, 90, 100). We suggest that these papers, or references cited in them, be consulted for these and related topics.

Usually, inbreeding refers to the mating of closely related individuals, and inbreeding depression is defined as reduced fitness of the offspring of these matings compared to the offspring of randomly mated individuals. In addition, though, genetic drift in small populations causing fixation of detrimental alleles can result in all, or nearly all, individuals in some populations having a lower fitness than other populations. Because populations of endangered species may be small in size or may have gone through bottlenecks or founder events, this effect may be particularly important in conservation. Within a population of an endangered species, inbred offspring may not have a lower fitness than non-inbred ones (we use "non-inbred" synonymously with randomly mated or outbred individuals), but instead all individuals have a lower fitness than the ancestral individuals before the effect of genetic drift. This type of inbreeding depression generally may be documented only by crossing to individuals from another population and observing the fitness of their progeny. Below we discuss a theoretical context for this phenomenon and describe three recent examples in which populations recovered fitness as the result of the introduction of outside individuals.

Model organisms can be useful in understanding underlying phenomena in biology (however, see 31), and this approach may determine unifying patterns in conservation genetics so that each species does not have to be considered as a unique case study (26, 28). This goal has recently been advanced by a number of laboratory experiments related to inbreeding depression using insects, including *Drosophila* (5, 6, 25, 28, 60, 61, 71), houseflies (11), flour beetles (78, 79), crickets (86), and butterflies (88). Many of the insights into inbreeding depression have come from detailed studies in *Drosophila* (14, 16, 18, 93).

It appears that in *Drosophila* approximately half the genetic load is from nearly recessive lethals and half from detrimentals of small effect but with higher dominance (14, 100). However, it is important to ask whether such findings are completely generalizable to endangered species. Most genetic phenomena, such as segregation, independent assortment, linkage, mutation, etc., are virtually identical across species, but *Drosophila* and endangered species may differ in some evolutionary and ecological factors that are important in inbreeding depression. For example, *Drosophila* have a very large effective population size, whereas in many endangered species, genetic drift is quite important either because of a currently small population size or because of past severe bottlenecks or founder events.

As a result, a smaller population (with greater genetic drift) may translate into a different genetic architecture of detrimental variation than that for a larger population. In a small, finite population, relative to a large, infinite population, lethals (and other variants of large detrimental effect) have a lower expected frequency

because selection will push them to a low frequency and then they will be lost from the population by genetic drift (17, 74). Similarly, the standing genetic variation in *Drosophila* may be larger than in endangered species and thereby prompt a pessimistic outlook on the amount of inbreeding depression and an optimistic perspective on adaptive potential. In addition, in a small, finite population, genetic drift becomes a stronger influence on allelic frequency than selection if $s < 1/2N$, where s is the selective disadvantage of homozygotes and N is the effective population size. In small populations, detrimental mutations with a selective disadvantage less than $1/2N$ become fixed much as if they were neutral (50, 58, 63, 66). As a result, fitness may decline over time, and the population may decrease in size so that detrimental mutants of larger effect become effectively neutral and subsequently are more likely to be incorporated. This feedback process has been named mutation meltdown and, in theory, may result in the extinction of small populations (65). It is not clear how significant mutation meltdown may actually be because extinction probability due to other factors may be high in such small populations (59); there are, however, examples of apparent fixation of deleterious variants in some endangered species with small population sizes.

IMPACT OF THE ENVIRONMENT ON LOWERED FITNESS IN INBRED INDIVIDUALS

The effects of inbreeding on endangered species have generally been examined in captive populations for which the environment may be less harsh than natural environments. For example, juvenile survival is generally much higher in captivity than it is in nature. Estimates of inbreeding depression from captivity or laboratory environments are thought to underestimate or at least to be different than the effects in a natural environment. Several studies have attempted to evaluate the effect of inbreeding on fitness in nature to determine the extent of this difference. It is difficult to have an appropriate experimental design in these instances, e.g., to replicate populations and have simultaneous controls (however, see 70a). Nevertheless, these experiments illustrate that inbreeding depression may have a significant effect on fitness in natural populations (see also 15a).

Jimenez et al (41) examined the survival of adult non-inbred and inbred white-footed mice (*Peromyscus leucopus noveboracensis*). Stock for the experiment was captured from the natural study site near Chicago, Illinois, brought into the laboratory, and bred to produce individuals with inbreeding coefficients of 0.00 or 0.25 (from full-sib matings). Almost 800 mice, nearly equally split between non-inbred and inbred, were released during three different periods. The area had a low number of mice during the release, suggesting that the environment was harsh because of some unknown cause. For the 10 weeks following release, non-inbred individuals had a higher weekly survivorship at all census times than the inbred individuals (Figure 1). Using capture-recapture data, the weekly survival of the
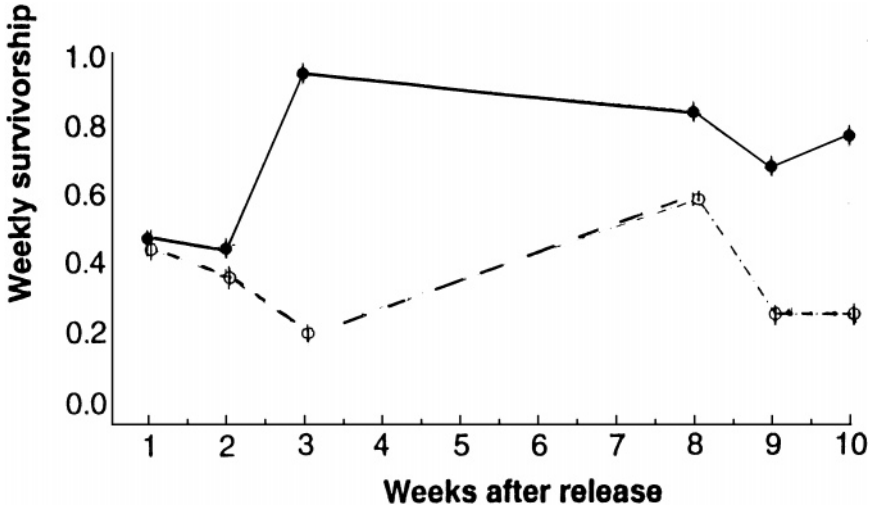
Figure 1 Weekly survivorship of non-inbred (solid line) and inbred (broken line) white footed mice over 10 weeks in a natural habitat (from 41).

inbred mice was estimated to be 56% that of the non-inbred mice. In addition, inbred male mice lost significant body mass throughout the experiment, while non-inbred male mice did not.

The population crash of the wild population of song sparrows (*Melospiza melodia*) on Mandarte Island, British Columbia, involves a documented case of inbreeding depression in the wild (49). This population appears to undergo periodic crashes, probably due to severe winter weather: The decline in 1989 killed 89% of the adult animals. Because there had been an extensive program to mark individuals in this population over several generations, the inbreeding coefficient for most individuals was known before the crash. After the crash, the inbreeding coefficient for the 10 survivors was 0.0065 (only three had known inbreeding), whereas the inbreeding coefficient for the 206 birds that died was significantly higher at 0.0312. All the birds with inbreeding coefficients of 0.0625 or higher (13% of the population) died during the crash. Later, high inbreeding depression was shown in the population (48), consistent with the differential survival between inbred and non-inbred individuals.

Saccheri et al (89) reported that the rate of extinction was negatively correlated with heterozygosity in 42 small populations of fritillary butterflies (*Melitaea cinzia*). Over the summer of 1996, seven of these populations went extinct. The heterozygosity, as determined by seven allozyme loci and one microsatellite locus, was significantly lower in the populations that went extinct than in the surviving populations. To avoid the confounding effect of population size on both heterozygosity and extinction, in their statistical analysis, Saccheri et al controlled for

TABLE 1 (*a*) A general way to predict the fitness of inbred individuals in a natural environment based on the relative fitness of inbreds in the captive environment and the relative fitness of non-inbreds in a natural environment and (*b*) an example using the observed relative survival in the white-footed field mouse (41). Here the observed survival of inbred mice in the natural environment is much less than that expected.

		Population	
	Environment	Non-inbred	Inbred
(a) General	Captive	1	$\overline{w}_I$
	Natural	$\overline{w}_N$	$\overline{w}_I \overline{w}_N$
(b) Mouse data	Captive	1	$\overline{w}_I = 0.935$
	Natural	$\overline{w}_N = 0.221$	$\overline{w}_I \overline{w}_N = 0.207$
			Observed $= 0.046$

the effect of ecological variables on population extinction. Laboratory studies indicate substantial inbreeding depression in this species from one generation of full-sib mating (74a, 89). This amount of inbreeding is probably similar to that in some isolated populations that may have been founded by a single female, a scenario confirmed by extinction risk differences in experimental inbred and outbred populations (74a).

To quantify the effects of inbreeding in nature, let us assume that the fitness, or a fitness component, in the captive or laboratory environment for non-inbred individuals is standardized to be unity. The fitness relative to this in the natural environment is $\overline{w}_N$ (the effect of the environment), and the fitness relative to that in the inbred population is $\overline{w}_I$ (the effect of inbreeding). If we assume that the effects of inbreeding and the environment are multiplicative, then the expected fitness of the inbred group in the natural environment is $\overline{w}_I \overline{w}_N$ (part *a* of Table 1). In part *b* of Table 1, the data from the white-footed mouse example are given (41); these show that the survival of non-inbreds in the natural environment relative to the survival of non-inbreds in captivity is 0.221, and the survival of inbreds in captivity relative to non-inbreds in captivity is 0.935. Therefore, the predicted survival of inbreds in nature is 0.207, but the actual observed survival is only 0.046, about 22% of that predicted, suggesting that inbreds in nature survive much more poorly than expected.

To demonstrate that inbred individuals are more affected by a natural environment than expected, it is not enough just to show that inbred individuals have lower fitness in a natural environment than in a captive one. For example, the approach given in Table 1 provides a method to compare the predicted joint effects of a natural or more stressful environment and inbreeding on fitness and the reduction actually observed. This can also be measured by comparing estimates of the number of lethal equivalents in the captive and the natural environments (see below).

Another approach is to test for a significant environment-inbreeding interaction component in analysis of variance.

The Gila topminnow provides an unusual example of an endangered species that can be bred and evaluated in captive situations with replicates, simultaneous controls, and the other attributes that make model organisms useful. The Gila topminnow is a widely cited example of the importance of genetic variation to conservation because a sample from Sharp Spring, Arizona, was found to have both more allozyme variation and a higher value of traits potentially related to fitness than a sample from Monkey Spring, Arizona (80, 99). Recent studies with highly variable loci demonstrate that fish from Monkey Spring have substantial genetic variation (38, 76), but more relevant to our discussion is that measures of traits related to fitness in fish from different populations were, unlike in the previous study, quite similar (91). The differences between these studies were not trivial, and Sheffer et al (91) found that fish from Sharp Spring had neither higher survival, nor less bilateral asymmetry, nor larger size than did fish from other populations. In addition, the Sharp Spring sample of wild-caught fish had higher fecundity than that from the Monkey Spring, but a sample from another site had somewhat higher fecundity levels still. These fecundity differences disappeared, however, in the next laboratory-raised generation.

Sheffer et al (91) concluded that the differences were likely attributable to the laboratory environment used by Quattro & Vrijenhoek (80) in New Jersey being more stressful than theirs in Arizona. For example, Monkey Spring individuals experienced eight times as much mortality over the first 12 weeks and ten times as much bilateral asymmetry in New Jersey as in Arizona. Obviously, ability to cope with stressors is important for endangered species, but in this case, the stressors in the New Jersey environment are unknown, and they are probably unrelated to any stressors that would be encountered in natural populations in Arizona. Differences in fitness between populations in a stressful laboratory environment suggest that it would be useful to determine if these differences are also present in natural environments or if they cause the same effect between non-inbreds and inbreds in natural environments.

Sheffer et al (92) also examined populations from the four major watersheds containing Gila topminnows for inbreeding or outbreeding effects on several traits potentially related to fitness. These laboratory studies produced no evidence of either inbreeding or outbreeding depression: there was generally high survival, similar body size, and little bilateral asymmetry for all the inbred and outbred matings. Similarly, no evidence appeared of inbreeding or outbreeding effects for fecundity or sex ratio except for the sample from Monkey Spring, which had highly female-biased sex ratios and low fecundity after one generation of inbreeding. No evidence of an increase in fitness appeared in crosses between populations, suggesting there was no evidence of fixation of different detrimental alleles over the populations. However, as discussed above, in more extreme situations as often encountered in natural habitats, fitness components may be more influenced than in laboratory situations.

In captive breeding programs of endangered species, substantial effort is often focused on maintaining these species and, as a result, husbandry, diet, understanding of behavior, etc. improve over time. As a result, survival tends to increase over time even as the inbreeding coefficient increases if the population was started from a small number of founders. For example, in both the Mexican and red wolf captive breeding programs (46), a significant increase occurred in the survival of animals over time (Figure 2). Concurrently, the inbreeding coefficient increased so that any inbreeding depression might be cancelled out by, or confounded with, the temporal increase in viability. Similar temporal changes in survival have been observed in other species (45, 47).

GENETIC RESTORATION OF POPULATIONS WITH LOW FITNESS

Populations of some endangered species have become so small that they have lost genetic variation and appear to have become fixed for deleterious genetic variants. To avoid extinction from this genetic deterioration, some populations may benefit from the introduction of individuals from related populations or subspecies for genetic restoration, i.e., elimination of deleterious variants and recovery to normal levels of genetic variation. Hedrick (34) developed one way to assess the potential positive and negative effects of introducing individuals from genetically diverse but geographically isolated populations into apparently inbred populations, in evaluating the then-proposed genetic restoration of the Florida panther through the introduction of Texas cougars.

Some of these results are illustrated in Figure 3. First, consider the expected change in fitness after introduction of a gene causing lower fitness in the endangered population (bottom line), where the relative fitnesses of genotypes A_1A_1, A_1A_2, and A_2A_2, are 1, 1, and 0.5, respectively. In this case, the endangered population is fixed for detrimental allele A_2 and the outside population is fixed for allele A_1. If there is 20% gene flow from outside in the first generation and 2.5% every generation thereafter, the fitness quickly improves; before 10 generations, it has approached the maximum possible for this gene. One concern about this approach is that any locally adapted alleles may be swamped by gene flow from outside. To examine this scenario, it was assumed that the fitnesses of the genotypes are 1, 1.2, and 1.2, respectively, the endangered population is fixed for a dominant advantageous allele, and the outside population is fixed for an allele that is disadvantageous in the environment of the endangered population. In this case, the fitness is only slightly reduced as the result of gene flow (top line), so the advantageous allele is able to maintain itself in spite of gene flow from the outside. If these two effects are combined (broken line), then the expected fitness increases over time and approaches the maximum before 10 generations. These findings appear to be generally robust to the effects of finite population size, variation in the level of dominance, and other factors (34).
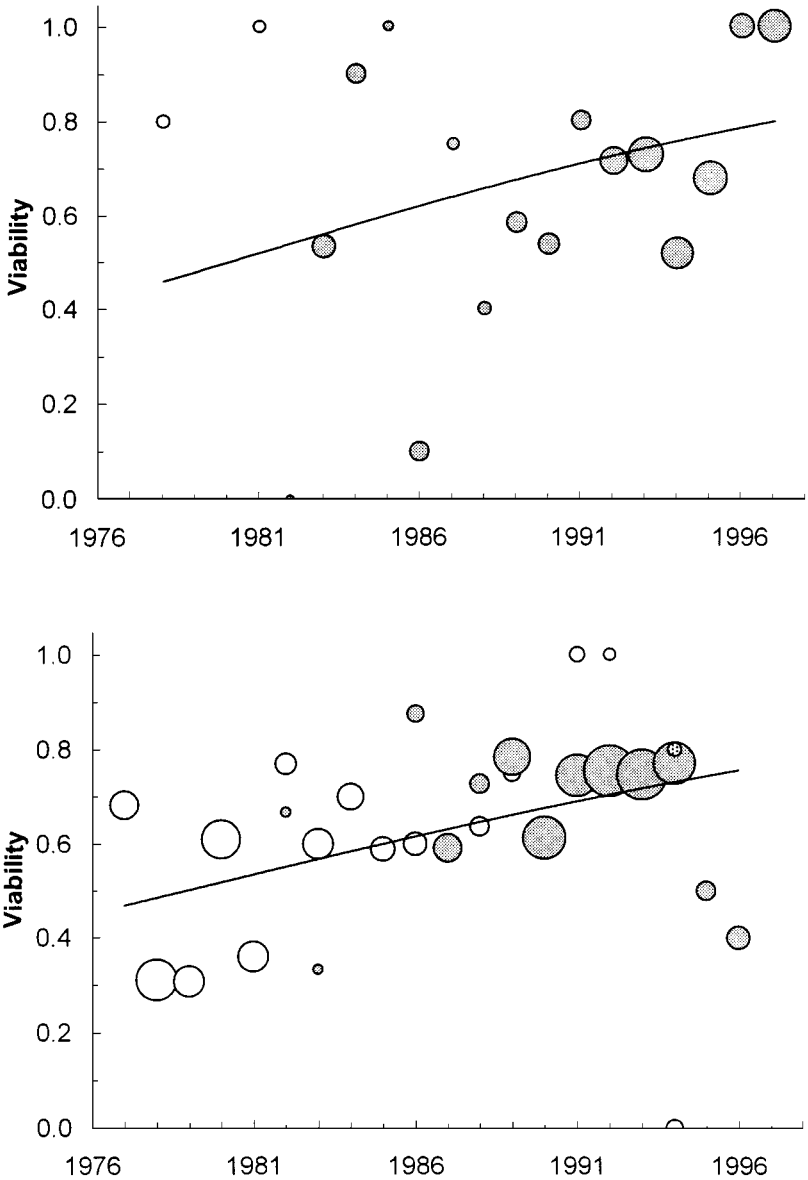
Figure 2 Viability of non-inbred individuals (open symbols) and inbred individuals (shaded symbols) for the Mexican (above) and red (below) wolves. The area of the circles is proportional to the number of individuals, and the fitted line shows the viability as a logistic function of year of birth (from 46).
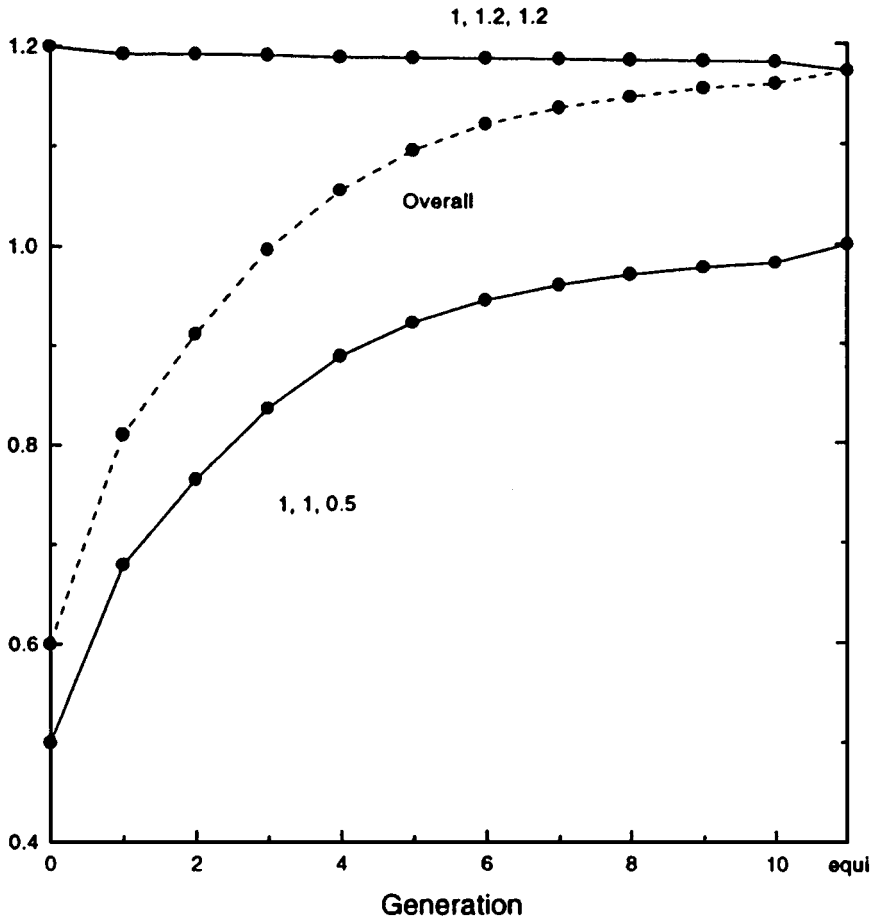
Figure 3   The change in relative fitness in a population over 10 generations with gene flow of 20% in the first generation and 2.5% per generation thereafter from an outside source, and with selection either causing lowered fitness (genotypes A_1A_1, A_1A_2, and A_2A_2 with fitnesses 1, 1, and 0.5) or an adaptive advantage (1, 1.2, 1.2), and the overall effect of these two loci (from 34).

Before we discuss several examples of apparently successful genetic restoration, several cautionary remarks are in order. If safeguards are not employed, introduction of animals into a population may have immediate detrimental effects on the population by the introduction of disease or other effects (94). If animals that have been in captivity are used for the introduction, they may be adapted to captivity and/or have lost adaptation to the natural environment. Further, introduction may potentially reduce the overall effective population size (36, 87), and outbreeding depression (in this case a lower fitness of the F_1s and future generations than that in the parental population) may further reduce the viability of the population.

Three experiments have suggested that gene flow from outside populations can restore the fitness of populations that seemed to be suffering from fixation of detrimental alleles. In these instances, the mean fitness of the F_1 individuals appears to be higher than that of individuals in the impacted population. Examination of inbreeding depression in these populations before infusion of outside individuals, using the traditional approach of comparing non-inbred and inbred offspring, may not reveal inbreeding depression because all individuals are similar genetically for detrimental alleles. Only by crossing to individuals from outside the population is the effect of inbreeding depression measurable and the fitness restored to a level found before the effect of genetic drift (14).

The last remaining population of the Florida panther (*Felis concolor coryi*) has a suite of traits that suggests genetic drift has fixed (or nearly fixed) the population for previously rare and potentially deleterious traits. These traits, which are found in high frequency only in the Florida panther and are unusual in other puma subspecies, include a high frequency of cryptochordism (unilateral undescended testicles), the poorest quality of semen recorded in any felid, kinked tail, and cowlick (85). In addition, a large survey of microsatellite loci have shown that Florida panthers have much lower molecular variation than other North American populations of mountain lions, and much lower variation today than found in samples from around 1900 (19). The Florida panther has been isolated in southern Florida since the early 1900s, and the effective population size in recent decades appears to be 25 or fewer. As a result, a program to release females from the closest natural population from Texas was initiated in 1995 to genetically restore fitness in this population (34). The introduced Texas females have bred with resident Florida panther males, and 14 F_1 offspring have been reproduced. Of these, none has a kinked tail and only one has a cowlick (57). Although the sample size is small, and cryptochordism and semen quality have not been evaluated, the frequency of detrimental traits appears to have been greatly reduced. This may become an important example of genetic restoration.

An isolated population of an adder (*Vipera berus*) in southern Sweden appears to have accumulated deleterious traits during a decline in population size to about 10 males in 1992 (70). During this decline, there was very low recruitment, a high proportion of deformed or stillborn offspring, and very low genetic variability. Twenty males from another population were captured and released for three years into the site. In 1996, the first year that new adult male adders were expected to be observed, there was increased recruitment of F_1 individuals (69). The number continued to increase and in 1999 32 male adders were observed, the most since 1981.

A remnant population of the greater prairie chickens (*Tympanuchus cupido pinnatus*) in Illinois provides another example of possible genetic restoration. The population decreased from around 2000 individuals in 1962 to fewer than 50 in 1994 (101). Fitness, as measured by fertility and hatching rates, declined over this period, and an estimate of genetic variation in the population was low compared both to populations from other states (7) and to historical specimens

from Illinois (8). In 1992, 271 birds were translocated from large populations in other states; nests monitored after the translocation suggested restored fertility and hatching rates.

MEASURING INBREEDING DEPRESSION

Several experimental and observational methods have been used to examine the effect of inbreeding on fitness and its components, and several statistical approaches have been used to quantify the extent of inbreeding depression. The simplest method compares the mean value of non-inbred individuals with the mean value of inbred individuals. This difference is usually standardized by expressing it as a proportional change in fitness $\delta = (\overline{w}_N - \overline{w}_I)/\overline{w}_N$. δ has both been called the coefficient of inbreeding depression and the cost of inbreeding. This statistic is only meaningful when defined so that $\overline{w}_I$ is expected to be less than $\overline{w}_N$ (e.g. measuring viability instead of mortality).

When data exist for multiple levels of inbreeding, the rate of decline in fitness with increased inbreeding is often of interest, which requires a model of how inbreeding affects fitness components. If loci causing inbreeding depression interact independently and additively, then fitness will decline linearly with increased inbreeding, as $\overline{w}_I = \overline{w}_N - bf$ where f is the inbreeding coefficient. The constant b can be used to compare the effects of inbreeding across variable levels of inbreeding but not across traits with different magnitudes. A standardized rate of decline of fitness, b', is obtained by dividing the rate at which fitness declines by the fitness of non-inbred individuals as $b' = b'/\overline{w}_N$ or, equivalently, by dividing the cost of inbreeding by how much inbreeding occurred, $b' = \delta/f$ (15). For example, if $\overline{w}_N = 80$ and $\overline{w}_I = 50$ for individuals with an inbreeding coefficient of 0.25 (full-sib or parent-offspring mating), then $\delta_{0.25} = 0.375$, $b = 120$, and $b' = 1.5$.

If loci determining fitness have independent, multiplicative effects, then fitness is expected to decline exponentially with inbreeding as $\overline{w}_I = \overline{w}_N e^{-Bf}$ so that where $B = -[\ln(\overline{w}_I/\overline{w}_N)]/f$ where B is a constant characteristic of the population for the given trait (14). This model is most useful for examining the relationship between inbreeding and viability, in which case $2B$ is approximately equal to the number of lethal equivalents affecting viability in a diploid genome (73). The lethal equivalent is a unit (although commonly used as a statistic) that can be used to quantify the effects of genes upon survival. One lethal equivalent is defined as a set of alleles that, if dispersed in different individuals, would, on average, be lethal in one individual of the group. For example, two alleles, which each cause death 50% of the time, constitute one lethal equivalent. The number of lethal equivalents in a diploid genome provides a measure of the potential effects of deleterious, recessive alleles.

A good example of the calculation of $2B$ is from the white-footed mouse study (41) in which the survival from birth to weaning at day 20 of non-inbred mice in captivity was 0.879 and survival of inbred mice ($f = 0.25$) was 0.822. Thus,

the estimated number of lethal equivalents in a diploid genome in juvenile mice in captivity is then 0.54. On the other hand, for the initial three-week release period in the natural environment, adult survival for non-inbreds was 0.194 and inbreds was 0.040. Using these data, the estimated number of lethal equivalents is 12.64 in the natural environment. This also illustrates the extreme environmental dependence of inbreeding depression in this case.

Although linear and exponential relationships between fitness and inbreeding are conceptually different, they predict similar response of fitness for low to moderate amounts of inbreeding. For example, if the cost of full-sib mating equals its median estimate among mammals of 0.32 (81), then both models predict a similar response of fitness to inbreeding from values of f less than 0.375.

In captive populations of endangered species, there are often various inbreeding categories, and generally least squares linear regression has been used to estimate B (81, 95). However, this approach does not work when there have been no survivors in a given inbreeding class because the logarithm of zero is undefined. As a result, a "small sample size correction" has been used to circumvent this problem (95), although this correction introduces a bias in the estimation procedure. To avoid this bias, Kalinowski & Hedrick (43) advocated using a maximum likelihood approach, which does not necessitate a small sample size correction.

An obstacle to measuring inbreeding depression in endangered species is the tendency for modern captive breeding programs to unintentionally reduce the statistical power to measure inbreeding depression (44). By preferentially pairing unrelated individuals, the distribution of inbreeding coefficients in a population that is managed to maximally preserve genetic variation will narrow until all individuals have approximately the same inbreeding coefficient. When this occurs, data from subsequent births will provide little additional information on the relationship between fitness and inbreeding.

Measuring the effect of inbreeding requires estimating inbreeding coefficients and a measure of fitness for a set of individuals. Estimating inbreeding coefficients for most populations is difficult because it generally requires knowing the pedigree of the individuals (1). Furthermore, calculating inbreeding coefficients from pedigrees requires specifying a degree of relatedness among founders of the pedigree. When no information is available, founders are usually assumed to be non-inbred and unrelated. If these assumptions are not true then inbreeding coefficients within the pedigree may be underestimated. Only captive populations generally have good enough pedigree information to calculate inbreeding coefficients. but in a few wild populations in which parents are known for several generations, good estimates of inbreeding coefficients have been obtained (49, 98).

Highly variable loci appear to provide an approach to estimating relatedness that does not require pedigree data (84). The heterozygosity at 29 microsatellite loci was determined in captive gray wolves with inbreeding coefficients known from pedigree information (24). The relationship of the average observed heterozygosity for these loci and f is given in Figure 4. The linear regression (solid line) explains 67% of the variation and is highly statistically significant. With this baseline,
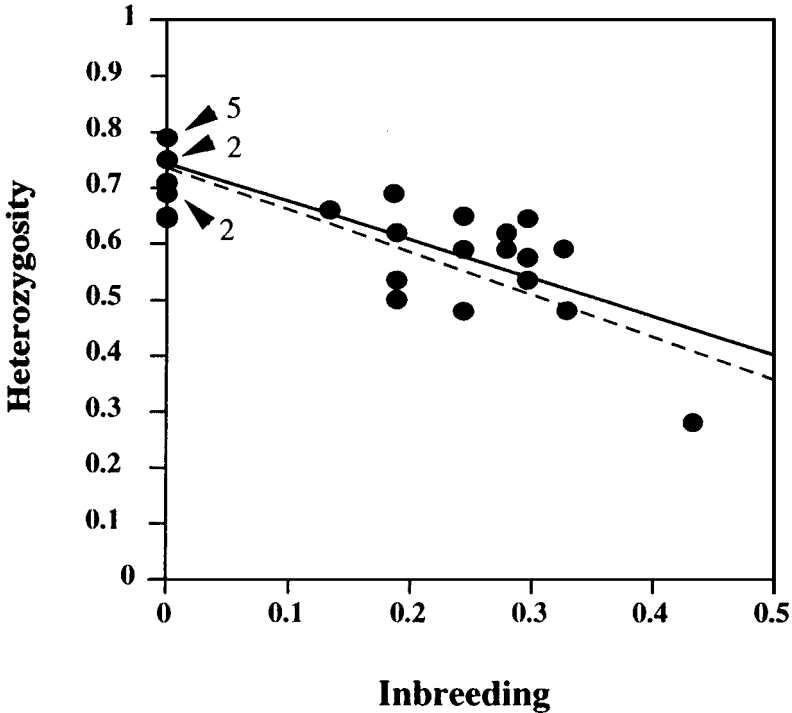
Figure 4   The relationship between individual average heterozygosity at 29 microsatellite loci in captive Scandinavian gray wolves. The straight line is the regression of individual heterozygosity on inbreeding coefficient, and the broken line indicates the expected relationship between H and f assuming that $H = 0.75$ between unrelated wolves (from 24).

Ellegren (24) evaluated the level of inbreeding in a sample of 13 of the 60 to 70 wild wolves left in Sweden. He found that the mean heterozygosity for the same loci in the wild wolves is 0.52, suggesting that their average inbreeding coefficient is around 0.2 to 0.3. In fact, one wild wolf had a heterozygosity of only 0.25, lower than the most inbred captive wolf examined. In addition, estimates of relatedness (67) of the wild wolves revealed pairs that appeared to be closely related, consistent with the suggestion that some of the wolves surveyed had high inbreeding coefficients.

Inbreeding depression may not be detected for several reasons, none of which indicates that inbreeding depression is absent from the population or species. First, the effects of inbreeding are generally examined in only one or a few components of fitness. Lack of inbreeding depression for juvenile survival does not mean there is no inbreeding depression for fecundity or mating success, or that different fitness components do not interact to reduce overall fitness. Second, often inbreeding

depression is examined in a captive situation in which the environment may be more benign, or at least different, than in a natural environment. As a result, there may be no detectable inbreeding depression in the given environment, but there may be an effect in a different or a natural environment. Third, there may not be the statistical power to detect biologically important inbreeding depression. This can be the result of either small sample size or the structure of the pedigree in which many individuals have similar inbreeding coefficients.

Kalinowski et al (46) found no detectable evidence for inbreeding depression in captive populations of Mexican and red wolves, although there is substantial evidence for inbreeding depression in the highly inbred Scandinavian wolves (54, 55). However, they were able to obtain substantial data for only two fitness-related traits, juvenile survival and litter size, and all the animals were in captive environments. Further, although the sample sizes for juvenile viability were 251 and 688 animals in the Mexican and red wolf samples, respectively, there was low statistical power to demonstrate the absence of inbreeding depression because of the structure of the pedigrees(44). It is important to report such "negative" results (along with their limitations), or the overall perspective of the extent and pattern of inbreeding depression may become biased. The potential presence of undocumented inbreeding depression in the Mexican wolf captive breeding program was an important motivation for combining the three independent (and inbred) lineages of Mexican wolves into one population (37). The inclusion of the two other lineages into the captive breeding program resulted in increased genetic diversity (by increasing the number of founders from 3 to 7) and may overcome any reduction of fitness that may have resulted from fixation of detrimental alleles within the lineages.

Interpretation of experimental results requires an understanding of the many statistical pitfalls associated with phenotypic data in inbred populations. The issues that should be addressed in experimental design and data analysis are complex, and it is important to be cautious in the analysis and interpretation of most data (see 68 for a detailed review). For example, the phenotype of individuals in multigenerational studies can be influenced by environmental trends. Plant studies may avoid this problem by storing seeds from each generation, then raising them contemporaneously in a common environment (4). When this approach is not possible, an alternative is to infer environmental effects by contemporaneously observing a large, randomly mating control population. With these data, the mean phenotype of inbred lineages can be adjusted to compensate for the changing effects of the environment. Another possible way to minimize this potential effect is to examine multiple levels of inbreeding in one environment at one time. In addition, statistical examination of phenotypes must account for the dependence of the phenotype of sequential samples upon their predecessors, decreasing variance for phenotypic traits caused by loss of genetic variation, and variation in the genetic comparison of the individuals that founded lineages. Lynch (62) maintains that these issues require a reliable study to have a large number of replicate populations.

PURGING INBREEDING DEPRESSION—EVIDENCE AND SIGNIFICANCE

After Ralls et al (82) documented inbreeding depression, there was a general effort to avoid inbreeding in the management of captive populations. However, inbreeding is unavoidable in populations founded from a small number of individuals as were many captive populations of endangered species. Templeton & Read (95) proposed to eliminate (purge) inbreeding depression through carefully controlled breeding and selection, a program that they claimed was successful in a captive population of the endangered Speke's gazelle. The original captive Speke's gazelle population was descended from three females and one male, which were; therefore, soon faced with unavoidable half-sib or parent-offspring matings. Mating pairs for the Speke's gazelle population were selected for three years; the second and third generations of inbred births had significantly higher viability than did the first generation of inbred gazelles. It appeared that inbred gazelles with inbred parents had higher survival than inbred gazelles with non-inbred parents, suggesting reduction of inbreeding depression because of past inbreeding. Although purging has not become an accepted strategy for managing small populations, the Speke's gazelle captive breeding program has remained a prominent case study in the inbreeding depression and conservation biology literature and is widely cited as a successful example (77).

Several authors have questioned Templeton & Read's evaluation on a variety of grounds (26, 33, 51, 105, but see 96). Most recently, Kalinowski et al (47) argued that the evidence for selection reducing inbreeding depression in the Speke's gazelle breeding program is based on a mischaracterization of when viability increased. Previous analyses compared the viability of the first generation of inbred births (born to non-inbred parents) with the viability of the second and third generations of inbreeding (born to non-inbred parents). These analyses assumed that the observed increase in viability occurred after selection had operated on the first generation of inbred gazelles. In contrast, Kalinowski et al (47) showed that the viability of inbred births actually increased during the first generation of inbreeding, before selection could have been detected. More specifically, inbred gazelles with no ancestral inbreeding born prior to 1976 had low viability, whereas similar gazelles born in 1976 or later had higher viability. In addition, the second generation of inbred gazelles (inbred gazelles with inbred parents), which could have benefited from selection in the previous generation, had a viability similar to the first generation of inbred gazelles born after 1975.

One way to illustrate this temporal change is to categorize gazelles by year of birth and parental inbreeding coefficient (Figure 5). The offspring of non-inbred gazelles born before 1976 (curve Ia) have significant inbreeding depression, but the offspring of non-inbred gazelles born in or after 1976 have no significant inbreeding depression (curve Ib). As reported by Templeton & Read (95), the offspring of inbred gazelles (curve II), most of which were born in or after 1976, do not have
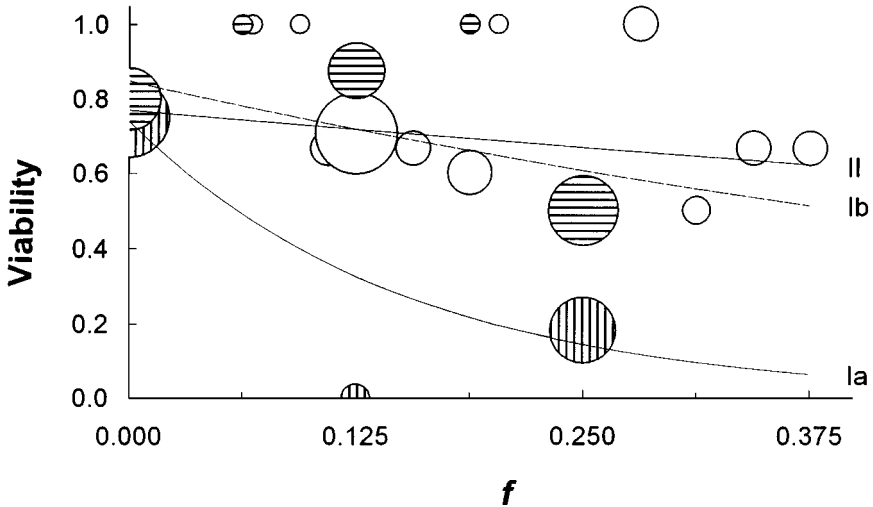
Figure 5   Observed (circles) and fitted (curved lines) viability in Speke's gazelles categorized by year of birth (before 1976, or in and after 1976) and parental inbreeding coefficient (non-inbred or inbred parents). Offspring of non-inbred gazelles born before 1976 (curve Ia, vertically lined circles), offspring of non-inbred gazelles born after 1976 (curve Ib, horizontally lined circles), and offspring of inbred gazelles (curve II, open circles) are given (from 47).

significant inbreeding depression. Thus, in the last half of the period examined, the offspring of neither non-inbred nor inbred gazelles exhibit inbreeding depression. In other words, inbred gazelles born in or after 1976 do not have reduced viability, whether or not their parents are inbred. In addition, simulation of the Speke's gazelle pedigree suggests that it is very unlikely that inbreeding depression could be eliminated in this short time period (47). In other words, it appears that the Speke's gazelle captive breeding program is a better example of the complexity of inbreeding depression, apparently related to unknown changes in the environment over time, than of purging. In either case, the Speke's gazelle captive breeding illustrates that populations with significant inbreeding depression can successfully be founded from a small number of individuals if their reproductive potential is large enough. Significantly, in a recent examination of the potential effects of purging in 17 mammalian species (2), a nonsignificant reduction in inbreeding depression in the updated Speke's gazelle captive population was found, and the inbreeding depression in the Speke's gazelle was the highest of any of species analyzed.

Since the Speke's gazelle captive breeding program fueled interest in purging as a conservation strategy, experimental work has examined the purging process in detail. Much of the work has centered on plants and has been thoroughly reviewed (12). Some experimental studies have closely examined the response of

inbreeding depression to multiple generations of selection, and in several insect model systems, there appears to have been a rebound in fitness (10, 25, 88). In a 10-generation study of inbreeding depression in *Peromyscus* (53), three subspecies differed in response. In general, fitness measures declined in each subspecies during the first generation of inbreeding. Subsequent generations of inbreeding were accompanied by improvements for one of the subspecies, another subspecies showed consistent decreases in fitness with subsequent inbreeding, and in the third subspecies, inbreeding depression was exacerbated by further inbreeding.

In endangered species that have low reproductive potential, deliberate inbreeding to purge inbreeding depression is a risky strategy because the added reduction in fitness from inbreeding may result in extinction. Even if the population survives, detrimental alleles may become fixed permanently, lowering the population fitness; genetic variation for other loci may be lost (33, 100). Minimizing inbreeding is the only currently accepted method for minimizing inbreeding depression. This can be accomplished in several related ways. The effective size of a population can be increased by increasing its census size or by minimizing any of the ways a real population departs from the Wright-Fisher ideal population (35). In addition, the typical genetic goal in management of captive populations has been to minimize loss of genetic variation (and inbreeding). Most modern captive breeding programs with pedigreed populations have adopted maximization of genetic diversity (measured by expected heterozygosity) as their primary genetic goal (3), with the implicit assumption that this will restrict inbreeding to an acceptable level. This is accomplished by selecting individuals to mate whose offspring minimize the average relatedness of the population.

CONCLUSIONS

Influence of inbreeding on fitness-related traits in endangered species and other organisms appears to be variable over populations, traits, and environments. Because endangered species generally have small population sizes and may have gone through bottlenecks, the genetic characteristics of inbreeding depression may differ in endangered species from more cocies, and they may have lower numbers of lethals contributing to the genetic load. Or, the effects of genetic drift may be present in only some endangered species, resulting in a greater variation over populations or species in the characteristics of inbreeding depression than in more common species.

The effects of inbreeding on fitness vary over species. Some of this variation is due to chance, including variation in number of lethal equivalents in the founders (52), but much of it may be due to different mean levels of inbreeding depression over the species, traits, or environments examined. For example, a low inbreeding coefficient of 0.03125 appeared to have an effect in houseflies (9), whereas in some experiments with *Drosophila*, an inbreeding coefficient of 0.7 was necessary to have an impact (27). Therefore, it is problematic to predict the expected effect of

inbreeding on fitness in an unexamined endangered species. However, the perspective provided here should give a context to evaluating inbreeding depression and suggest that its effects are generally likely to be more than first observed, rather than less.

Unless there is a high genetic load, detecting inbreeding depression in endangered species may be difficult in both natural and captive environments because of low statistical power, relatively benign conditions in captive environments, and inability to examine all aspects of fitness. Therefore, even if there is no statistical evidence for inbreeding depression, it is prudent to assume it is present. If it is assumed not to be present, then inbreeding may result in a loss of fitness that could have been avoided. On the other hand, just because there is inbreeding depression or low fitness because of past fixation from genetic drift, one should not give up on the population. For populations with low fitness, recent examples have shown that introduction of individuals from outside can genetically restore the fitness of the population. For populations with high inbreeding depression, sometimes alleles with large detrimental effects may be purged both in theory (33, 100) and in laboratory experiments with model insects. However, the oft-cited example of reduced inbreeding depression in the Speke's gazelle is most parsimoniously explained by a change in survival of inbred animals over time.

One of the early guidelines in captive breeding of endangered species to avoid inbreeding depression was based on the observation that "animal breeders accept inbreeding coefficients as high as a one percent increase per generation (i.e. an effective population size of 50) in domestic animals without great concern" (29). It was assumed that such slow inbreeding allows selection to remove deleterious alleles without endangering the population. However, a number of endangered species with successful breeding programs have had quite low founder numbers, e.g. Speke's gazelle with 4, Przewalski's horse with 13, black-footed ferret with 6, etc. Just because the numbers are low does not mean we should not make all attempts to save a species. All the surviving individuals have the unique characteristics of the species, and eventually the population number may be high enough to overcome the detrimental effects of the initial population size restriction. However, Wang et al (100), who have explored theoretically the effects of the nature of the mutational load, reproductive capacity, and the organization of the genome, demonstrate that when the effective size is 50, fitness generally declines because of genetic fixation of detrimental alleles.

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LITERATURE CITED

1. Ballou JD. 1983. Calculating inbreeding coefficients from pedigrees. In *Genetics and Conservation*, ed. CM Schonewald-Cox, SM Chambers, B MacBryde, L Thomas, pp. 509–20. Menlo Park, CA: Benjamin/Cummings

2. Ballou JD 1997. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *J. Hered.* 88:169–78

3. Ballou JD, Gilpin M, Foose TJ, eds. 1995. *Population Management for Survival and Recovery.* New York: Columbia Univ. Press

4. Barrett SCH, Charlesworth D. 1991. Effect of a change in the level of inbreeding on the genetic load. *Nature* 352:522–24

5. Bijlsma R, Bundgaard J, Van Putten WF. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* 12:1125–37

6. Bijlsma R, Bundgaard J, Boerema AC. 2000. Does inbreeding affect the extinction risk of small populations? Predictions from *Drosophila*. *J. Evol. Biol.* 13:502–14

7. Bouzat JL, Cheng HH, Lewin HA, Westemeier RL, Brawn JD, Paige KN. 1998. Genetic evaluation of a demographic bottleneck in the greater prairie chicken. *Conserv. Biol.* 12:836–43

8. Bouzat JL, Lewin HA, Paige KN. 1998. The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. *Am. Natur.* 152:1–6

9. Bryant EH, McCommas SA, Combs LM. 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. *Genetics* 114:1191–1211

10. Bryant EH, Meffert LM, McCommas SA. 1990. Fitness rebound in serially bottlenecked populations of the house fly. *Am. Natur.* 136:542–49

11. Bryant EH, Backus VL, Clark ME, Reed DH. 1999. Experimental tests of captive breeding for endangered species. *Conserv. Biol.* 13:1487–96

12. Byers DL, Waller DM. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.* 30:479–513

13. Charlesworth B. 1998. The effect of synergistic epistasis on the inbreeding load. *Genet. Res.* 71:85–89

14. Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237–68

14a. Charlesworth D, Charlesworth B. 1999. The genetic basis of inbreeding depression. *Genet. Res.* 74:329–40

15. Charlesworth B, Hughes KA. 1999. The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics from Molecules to Morphology*, ed. RS Singh, CB Krimbas CB, pp. 369–92. Cambridge, UK: Cambridge Univ. Press

15a. Coltman DW, Pilkington JG, Smith JA, Pemberton JM. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259–67

15b. Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity* 83:260–70

16. Crow JF. 1993. Mutation, mean fitness and genetic load. *Oxford Rev. Evol. Biol.* 9:3–42

17. Crow JF, Kimura M. 1970. *An Introduction to Population Genetics Theory.* New York: Harper & Row

18. Crow JF, Simmons MJ. 1983. The mutation load in Drosophila. In *The Genetics and Biology of Drosophila.* Volume 3c,

ed. M Ashburner, HL Carson, JN Thompson, pp. 1–35. London: Academic Press

19. Culver M, Johnson WE, Pecon-Slattery J, O'Brien SJ. 2000. Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* 91:186–97

20. Darwin CR. 1868. *Variation of Animals and Plants under Domestication.* London: John Murray

21. Darwin CR. 1876. *The Effects of Cross and Self fertilization in the Vegetable Kingdom.* London: John Murray

22. DeRose, MA, Roff DA. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* 53:1288–92

23. Dudash MR, Carr DE. 1998. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* 393:682–84

24. Ellegren H. 1999. Inbreeding and relatedness in Scandinavian grey wolves Canis lupus. *Hereditas* 130:239–44

25. Fowler K, Whitlock MC. 1999. The variance in inbreeding depression and the recovery of fitness in bottlenecked populations. *Proc. R. Soc. Lond B.* 266:2061–66

26. Frankham R. 1995. Conservation genetics. *Annu. Rev. Genet.* 29:305–27

27. Frankham R. 1995. Inbreeding and extinction: a threshold effect. *Conserv. Biol.* 9:792–99

28. Frankham R. 1999. Resolving conceptual issues in conservation genetics: the roles of laboratory species and meta-analyses. *Hereditas* 130:195–201

29. Franklin IR. 1980. Evolutionary change in small populations. In *Conservation Biology: An Evolutionary-Ecological Perspective*, ed. ME Soule, BA Wilcox, pp. 135–49. Sunderland, MA: Sinauer

30. Fu Y-B, Namkoong G, Carlson JE. 1998. Comparison of breeding strategies for purging inbreeding depression via simulation. *Conserv. Biol.* 12:856–64

31. Harshman LG, Hoffmann AA. 2000. Laboratory selection experiments using *Drosophila*: What do they really tell us? *Trends Ecol. Evol.* 15:32–36

32. Hedrick PW. 1987. Genetic bottlenecks. *Science* 237:963

33. Hedrick PW. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73:363–72

34. Hedrick PW. 1995. Gene flow and genetic restoration: the Florida panther as a case study. *Conserv. Biol.* 9:996–1007

35. Hedrick PW. 2000. *Genetics of Populations.* Boston: Jones & Bartlett. 2nd ed.

36. Hedrick PW, Hedgecock D, Hamelberg S. 1995. Effective population size in winter-run chinook salmon. *Conserv. Biol.* 9:615–24

37. Hedrick PW, Miller PS, Geffen E. Wayne R. 1997. Genetic evaluation of the three captive Mexican wolf lineages. *Zoo Biol.* 16:47–69

38. Hedrick PW, Parker KM. 1998. MHC variation in the endangered Gila topminnow. *Evolution* 52:194–99

39. Hedrick PW, Savolainen O, Karkkainen K. 1998. Factors influencing the extent of inbreeding depression: an example from Scots pine. *Heredity* 82:441–50

40. Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70

41. Jimenez JA, Hughes KA, Alaks G, Graham L, Lacy RC. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* 266:271–73

42. Johnston MO, Schoen DJ. 1995. Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* 27:226–29

43. Kalinowski ST, Hedrick PW. 1998. An improved method for estimating inbreeding depression in pedigrees. *Zoo Biol.* 17:481–97

44. Kalinowski ST, Hedrick PW. 1999. Detecting inbreeding depression is difficult in captive endangered species. *Anim. Conserv.* 2:131–36

45. Kalinowski ST, Hedrick PW. 2000. Inbreeding depression in captive bighorn sheep. *Zoo Biol.* Submitted

46. Kalinowski ST, Hedrick PW, Miller PS. 1999. No evidence of inbreeding depression in Mexican and red wolves. *Conserv. Biol.* 13:1371–77

47. Kalinowski ST, Hedrick PW, Miller PS. 2000. A close look at inbreeding depression in the Speke's gazelle captive breeding program. *Conserv. Biol.* In press

48. Keller, LF. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* 52:240–50

49. Keller LF, Arecese P, Smith JMN, Hochachka WM, Stearns SC. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature* 372:356–57

50. Kimura M. *The Neutral Theory of Molecular Evolution.* Cambridge, UK: Cambridge Univ. Press

51. Lacy RC. 1997. Importance of genetic variation to the viability of mammalian populations. *J. Mammal.* 78:320–35

52. Lacy RC, Alak G, Walsh A. 1996. Hierarchial analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution* 50:2187–2200

53. Lacy RC, Ballou JD. 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus*. *Evolution* 50:2187–2200

54. Laikre L, Ryman N. 1991. Inbreeding depression in a captive wolf (*Canis lupus*) population. *Conserv. Biol.* 5:33–41

55. Laikre L, Ryman N, Thompson ES. 1993. Hereditary blindness in a captive wolf population: frequency reduction of a deleterious allele in relation to gene conservation. *Conserv. Biol.* 7:592–601

56. Laikre L. 1999. Conservation genetics of Nordic carnivores: lessons from zoos. *Hereditas* 130:203–16

57. Land D, Lotz M, Shindle D, Taylor SK. 1999. Florida panther genetic restoration and management:Annual performance report 1998–1999. Florida Fish & Wildlife Conservation Commission, Naples, FL

58. Lande R. 1994. Risk of population extinction from fixation of new deleterious mutation. *Evolution* 48:1460–69

59. Lande R. 1995. Mutation and conservation. *Conserv. Biol.* 9:782–91

60. Latter BDH. 1998. Mutant alleles of small effect are primarily responsible for the loss of fitness with slow inbreeding in *Drosophila melanogaster*. *Genetics* 148:1143–58

61. Latter BDH, Mulley JC, Reid D, Pascoe L. 1995. Reduced genetic load revealed by slow inbreeding in *Drosophila melanogaster*. *Evolution* 139:287–97

62. Lynch M. 1988. Design and analysis of experiments on random drift and inbreeding depression. *Genetics* 120:791–807

63. Lynch M. 1996. A quantitative-genetic perspective on conservation issues. In *Conservation Genetics*, ed. J Avise, J Hamrick, pp. 471–501. New York: Chapman & Hall

64. Lynch M, Blanchard J, Houle D, Kibota T, Schultz S, Vassilieva L, Willis J. 1999. Perspective: spontaneous deleterious mutation. *Evolution* 53:645–63

65. Lynch, M, Conery J, Burger R. 1995. Mutation meltdowns in sexual populations. *Evolution* 49:1067–88

66. Lynch M, Gabriel W. 1990. Mutation load and the survival of small populations. *Evolution* 44:1725–37

67. Lynch M, Ritland K. 1999. Estimation of pairwise relatedness with molecular markers. *Genetics* 152:1753–66

68. Lynch M, Walsh B. 1998. *Genetics and Analysis of Quantitative Traits.* Sunderland, MA: Sinauer

69. Madsen T, Shine R, Olsson M, Wittsell H. 1999. Restoration of an inbred adder population. *Nature* 402:34–35

70. Madsen T, Stille B, Shine R. 1996. Inbreeding depression in an isolated population of adders *Vipera berus*. *Biol. Conserv.* 75:113–18

70a. Meagher S, Penn DJ, Potts WK. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci USA* 97:3324–29

71. Miller PS, Hedrick PW. 1993. Inbreeding and fitness in captive populations: lessons from *Drosophila*. *Zoo Biol.* 12:333–51

72. Mills LS, Smouse PE. 1994. Demographic consequences of inbreeding in remnant populations. *Am. Nat.* 144:412–31

73. Morton NE, Crow JF, Muller HJ. 1956. An estimate of the mutational damage in man from data on consanguineous marriages. *Proc. Natl. Acad. Sci. USA.* 42:855–63

74. Nei M. 1968. The frequency distribution of lethal chromosomes in finite populations. *Proc. Natl. Acad. Sci. USA* 60:517–24

74a. Nieminen M, Singer MC, Fortelius W, Schops K, Hanski I. 2000. Experimental confirmation of inbreeding depression increasing extinction risk in butterfly populations. *Am. Nat.* In revision

75. O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, et al. 1985. Genetic basis for species vulnerability in the cheetah. *Science* 227:1428–34

76. Parker KM, Sheffer RJ, Hedrick PW. 1999. Molecular variation and evolutionarily significant units in the endangered Gila topminnow. *Conserv. Biol.* 13:108–16

77. Pennisi E. 1999. The perils of genetic purging. *Science* 285:193

78. Pray LA, Goodnight CJ. 1995. Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum*. *Evolution* 49:176–88

79. Pray LA, Schwartz, JM, Goodnight CJ, Stevens L. 1994. Environmental dependency of inbreeding depression: implications for conservation biology. *Conserv. Biol.* 8:562–68

80. Quattro JM, Vrijenhoek RC. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. *Science* 245:976–78

81. Ralls K, Ballou JD, Templeton AR. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conserv. Biol.* 2:185–93

82. Ralls K, Brugger K, Ballou J. 1979. Inbreeding and juvenile mortality in small populations of ungulates. *Science* 206:1101–03

83. Ritland K. 1996. Inferring the genetic basis of inbreeding depression in plants. *Genome* 39:1–8

84. Ritland K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. *Genet. Res.* 67:175–85

85. Roelke ME, Martenson JS, O'Brien SJ. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* 3:340–50

86. Roff DA. 1998. Effects of inbreeding on morphological and life history traits of the sand cricket, *Gryllus firmus*. *Heredity* 81:28–37

87. Ryman N, Laikre L. 1991. Effects of supportive breeding on the genetically effective population size. *Conserv. Biol.* 5:325–29

88. Saccheri I, Brakefield PM, Nichols RA. 1996. Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution* 50:2000–13

89. Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–94

90. Schultz ST, Willis JH. 1995. Individual variation in inbreeding depression: the roles of inbreeding history and mutation. *Genetics* 141:1209–23

91. Sheffer RJ, Hedrick PW, Minckley WL, Velasco AL. 1997. Fitness in the endangered Gila topminnow. *Conserv. Biol.* 11:162–71

92. Sheffer RJ, Hedrick PW, Velasco A. 1999. Testing for inbreeding and outbreeding depression in the endangered Gila topminnow. *Anim. Conserv.* 2:121–29

93. Simmons MJ, Crow JF. 1977. Mutation affecting fitness in Drosophila populations. *Annu. Rev. Genet.* 11:49–78

94. Snyder NH, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, et al. 1996. Limitation of captive breeding in endangered species recovery. *Conserv. Biol.* 10:338–48

95. Templeton AR, Read B. 1983. The elimination of inbreeding depression in a captive herd of Speke's gazelle. In *Genetics and Conservation*, ed. CM Schonewald-Cox, SM Chambers, B MacBryde, L Thomas, pp. 41–61. Menlo Park, CA: Benjamin/Cummings

96. Templeton AR, Read B. 1998. Elimination of inbreeding depression from a captive population of Speke's gazelle: validity of the original statistical analysis and confirmation by permutation testing. *Zoo Biol.* 17:77–98

97. Uyenoyama MK, Holsinger KE, Waller DM. 1993. Ecological and genetic factors directing the evolution of self-fertilization. *Oxford Surv. Evol. Biol.* 9:327–81

98. Van Noordwijk AJ, Scharloo W. 1981. Inbreeding in an island population of the great tit. *Evolution* 35:674–88

99. Vrijenhoek RC, Douglas ME, Meffe GK. 1985. Conservation genetics of endangered fish populations in Arizona. *Science* 229:400–2

100. Wang J, Hill WG, Charlesworth D, Charlesworth B. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* 74:165–78

101. Westemeier, RL, Brown JD, Simpson SA, Esker TL, Jansen RW, et al. 1998. Tracking the long-term decline and recovery of an isolated population. *Science* 282:1695–98

102. Wielebnowski N. 1996. Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. *Zoo Biol.* 15:353–69

103. Willis JH. 1999. Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. *Genetics* 153:1885–98

104. Willis JH 1999. The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* 53:1678–91

105. Willis K, Wiese RJ. 1997. Elimination of inbreeding depression from captive populations: Speke's gazelle revisited. *Zoo Biol.* 16:9–16