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Inbreeding is known to lead to decreased survival and reproduction in captive populations of animals. It is also important to know whether inbreeding has deleterious effects in natural habitats. An estimate was made of the effects of inbreeding in white-footed mice, *Peromyscus leucopus noveboracensis*, derived from a wild population. This study demonstrates that inbreeding had a significant detrimental effect on the survivorship of mice reintroduced into a natural habitat. This effect was more severe than the effect observed in laboratory studies of the population.

Inbreeding adversely affects captive animals in a number of ways (*1–5*). However, captive animals are not exposed to many of the causes of mortality afflicting natural populations, such as predation, weather extremes, food stress, and epidemic disease. Deleterious effects of inbreeding have been demonstrated in natural populations of plants (6) and a few species of invertebrates (*7*) and fish (*8*), but the mobility and long generation lengths of mammals and other tetrapods have made it difficult to estimate the effect of inbreeding on survivorship in natural populations of these animals (*5*). This difficulty has led some researchers to question whether estimates of inbreeding depression made in captive populations can be used to predict the effects of inbreeding in nature or, indeed, whether natural populations experience inbreeding depression at all (*9*). Worldwide habitat destruction has forced many formerly natural populations into captivity for survival [such as the black-footed ferret *Mustela nigripes* (*10*)], and some captive populations have been inbred by necessity (*11*). The continued survival of many species depends on captive propagation before reintroduction (*12*), but inbreeding may compromise the fitness of reintroduced animals (*7*).

We developed a mark-release-recapture experiment to measure the effects of inbreeding on survivorship in a natural habitat. White-footed mice, *Peromyscus leucopus noveboracensis*, were collected from an area in which they were abundant and were used to found a laboratory population (*13*). Inbred and noninbred descendants of the wild-caught mice were released back into the field site from which the progenitors had been captured (Fig. 1) and were surveyed by trapping (*14*). A census of the field site in 1990 before the release resulted in only three captures during 1350 trap nights (0.002 mice per trap night). In 1988, when the progenitors of the laboratory population were collected, 0.163 mice per trap night had been captured by the same trapping protocol, suggesting that the population density had decreased. This decrease in population density may have been a direct result of the collection of the founders of the laboratory population but was more likely a result of some other, unknown process. This situation provided an opportunity to measure the effects of inbreeding in a natural setting that would allow new animals to become established without strong competition from an existing resident population (*15*).

Of the 786 animals released, 123 (15.7%) were recaptured at least once (Table 1). Mice were recaptured up to eight times for a total of 170 recaptures. Some were recaptured as long as 127 days after release, suggesting that many of the laboratory-bred mice became successfully established in the natural habitat. The low capture rate for wild mice can partially explain the high recapture rate for lab-reared animals. With few resident mice to force dispersal from the site, the reintroduced population quickly became established. Recapture histories for individual mice are available over the Internet (*16*) or from the authors.

Movement did occur among trapping areas and probably into nontrapped areas of the field site as well (Fig. 1). Most mice were recaptured within 50 m of the site of release. Of the 29 mice recaptured on a different trapline from their release site, 10 males and 6 females were inbred and 7 males and 6 females were noninbred. There is no significant effect of sex and inbreeding status on the tendency to move between areas (goodness of fit test based on log-linear models: $G = 1.464$, $P > 0.4$, with 2 df and expected frequencies calculated from the recapture data in Table 1). Thus, inbred mice do not move among the three release sites at a significantly

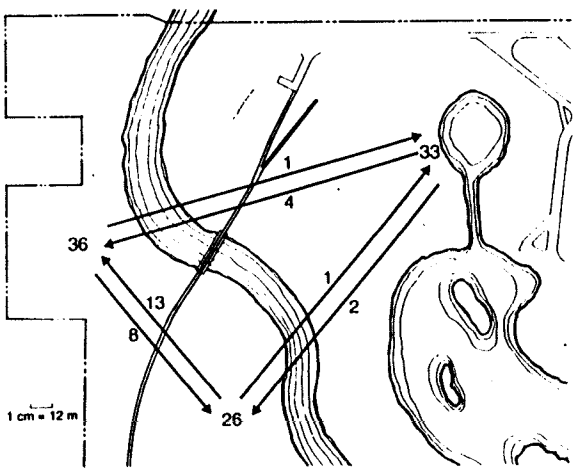

Fig. 1. Map of field site. Numbers at the ends of arrows represent the number of mice that were recaptured in the same area in which they were originally released. Numbers on directional arrows represent mice that were recaptured in a different area from the one in which they were released. Numbers on the figure sum to 124 because one mouse was recorded in all three areas. The field site comprised nonpublic areas of the Chicago Zoological Park, Brookfield, Illinois. All three areas are within a mixed deciduous forest. The broken line represents a fenced boundary between forested and nonforested areas. Wavy lines are water-depth contours. An old railroad bed and wooden bridge are indicated by solid lines.

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different rate than do noninbred mice.

We tested for an effect of inbreeding on survival by two different methods: a repeated-measures analysis of variance (ANOVA) of survivorship estimates and a generalized maximum likelihood estimation procedure for mark-recapture data. In the first method, Jolly-Seber estimates of survivorship were computed from release-recapture data on inbred and noninbred mice (Fig. 2). Repeated measures ANOVA revealed a significant effect of inbreeding on the survivorship of mice, with the weekly survivorship of inbred mice 56% that of noninbred mice on average (*17*) (Fig. 2). The effects on weekly survival of sex and time since release were not significant (*17*).

The effects of inbreeding on the amount of body mass lost between the time of release and the time of first recapture was also tested. We treated the mass lost by each mouse during this period (which varied in length among mice) as the dependent variable, inbreeding status as a fixed effect, and time between release and first recapture as a covariate in a separate analysis of covariance for each sex. Inbred males lost body mass throughout the experiment, whereas noninbred males regained body mass lost in the first few days after release [the regression coefficient of mass change on the time to first recapture for inbred males is -0.108 ± 0.077 (SE) g/day; the coefficient for noninbred males is 0.055 ± 0.029; the test of the effect of the interaction between inbreeding status and time to first recapture was $F_{(1,57)} = 4.789$ and $P = 0.03$]. In females, change in mass upon release was not affected by inbreeding [the regression coefficient for inbreds is 0.005 ± 0.033, and that for noninbreds is 0.046 ± 0.062, $F_{(1,57)} = 0.639$, $P = 0.43$]. Inbred mice did not differ significantly in body mass from noninbred mice at the time of release [159 inbred females averaged 21.8 g, 207 noninbred females averaged 21.4 g [$F_{(1,364)} = 0.47$, $P = 0.49$], 208 inbred males averaged 25.9 g, and 212 noninbred males averaged 25.0 g [$F_{(1,418)} = 2.26$, $P = 0.13$]]. At 24.1 g, the release-day body mass of males that were subsequently recaptured was somewhat lower, on average, than that for males that were never recaptured (25.7 g), but the difference was not significant [$F_{(1,418)} = 3.65$, $P = 0.06$]. There was no significant difference in body mass at release between females that were later recaptured (21.2 g) and those that were never recaptured (21.6 g) [$F_{(1,364)} = 0.343$, $P = 0.54$].

The maximum likelihood analysis allowed us to test assumptions of equal ease of capture of inbred and noninbred mice and to relax the assumptions of normal-distribution theory inherent in ANOVA approaches. We used the general estimation program SURGE (*18*) to calculate iteratively the maximum-likelihood estimates of survival and recapture probabilities and to perform likelihood ratio tests (LRT) of significance. These tests revealed a significant deleterious effect of inbreeding on survival and no significant difference in recapture rates for surviving inbred and noninbred individuals (*19*).

Significant differences in survivorship cannot be attributed to differential emigration of inbred versus noninbred mice (Fig. 1). The data on body mass at release indicate that inbred mice were in as good a condition as were noninbred mice when the experiment began, so inbred mice did not suffer high mortality by entering the release population at a disadvantage with respect to fat reserves.

To determine whether this population of *Peromyscus* was unusually sensitive to the effects of inbreeding, we computed lethal equivalents from the observed mortality rates of mice that were maintained in the lab. Lethal equivalents are calculated from the regression of survivorship on the inbreeding coefficient (*20*) and are used as a measure of the severity of inbreeding depression. The range of lethal equivalents with respect to juvenile survivorship for captive mammalian populations has been estimated as -1.36 to 30.32, with a median value of 3.14 per diploid genome (*4*). For rodents, the reported range is -0.14 to 15.2, with a median value of 1.15 (*4*). For the *Peromyscus* population used in the release experiment, we estimated 0.45 lethal equivalents per diploid genome for survival in the lab from birth to weaning at 20 days of age (survivorship of 188 noninbred mice = 0.879 ± 0.022, survivorship of 146 inbred mice = 0.822 ± 0.028). Apparently, the severity of inbreeding depression experienced by this population in a captive environment is low or moderate when compared to species of rodents and other mammals for which similar calculations have been made.

The calculated regression of the 3-week survival of released adult mice suggests that the inbreeding load for adult survival in a natural habitat [12.64 lethal equivalents, calculated from a mean survival estimate of 0.194 ± 0.126 for six groups of noninbred mice (males and females from three release batches) and a mean survival estimate of 0.040 ± 0.082 for six groups of inbred mice] was much larger than that for juvenile survival in the laboratory. This result suggests either that the inbreeding load in natural habitats is more severe than in the lab or that the load is greater in adults than in juveniles. However, the mortality rate in the laboratory for mice of the same age as the reintroduced mice is low for both inbred and noninbred individuals (for example, of 1561 mice weaned in the lab, 4 died in the 3 weeks after weaning). Greater severity of inbreeding depression in natural environments than in laboratory environments has also been reported in land snails (*7*).

We have shown that inbreeding is related to survivorship in a population that has been reintroduced to a natural habitat, with survivorship reduced for inbred mice. This result, together with the continual weight loss in inbred male mice after release, sug-

Table 1. Sample sizes of mice released and recaptured, classified by sex and inbreeding status. The proportions of mice that were recaptured did not differ between male and female mice ($\chi^2 = 0.084$, 1 df, $P = 0.77$) or between inbred and noninbred mice ($\chi^2 = 0.058$, 1 df, $P = 0.81$). Differences in survivorship between inbred and noninbred mice are revealed by analysis of Jolly-Seber survivorship estimates (*17*, *19*). There were seven male and two female wild mice captured.

Sex	Inbred	Noninbred	Total
	Mice released		
Male	208	212	420
Female	159	207	366
Total	367	419	786
	Mice recaptured		
Male	32	32	64
Female	24	35	59
Total	56	67	123

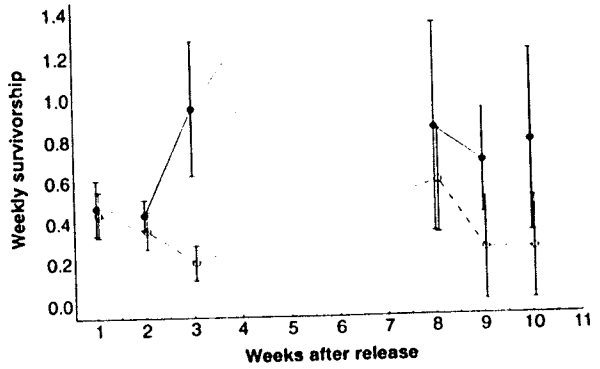

Fig. 2. Survivorship of inbred and noninbred mice over 10 weeks. Solid diamonds represent the mean survivorship values for noninbred mice and open circles represent values for inbred mice. Bars represent standard errors of the estimates. Symbols and error bars are slightly offset to show the area of overlap. Noninbred animals had higher survivorship than inbred animals during all six time intervals. The ratio of inbred survivorship to noninbred survivorship is 0.558 ± 0.121, averaged over the six estimates shown. None of the survivorship estimates from single time periods differ significantly between inbred and noninbred individuals. When the estimates are used as repeated measures of survivorship for groups of inbred and noninbred individuals, the overall difference is statistically significant [see (*17*)].

gests that this population is adversely affected by inbreeding when released into a natural habitat. The deleterious effects of inbreeding, as measured by lethal equivalents, were much more severe in the natural environment than in the captive environment. These results have been obtained for a population in which, compared to other mammalian populations, the effects of inbreeding in captivity are not particularly severe. Such experiments that are conducted in a natural environment are needed to address concerns about the applicability to natural populations of laboratory-based estimates of the effects of inbreeding. Unfortunately, the fate of many populations lies in people's ability to propagate them in captivity and then reintroduce them into a habitat representative of that of their ancestors. Even if the period of captivity is brief, inbreeding and other consequences of captive management can have a profound effect on the success of reintroduction efforts.

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13. The ancestors of the released mice had been collected from the release site in Cook County, IL, and from nearby sites in Lake County (95% were captured in Cook County and 5% in Lake County). All captive mice were housed in a single room maintained at 21° ± 1°C, with 15 air exchanges per hour and a controlled (12-hour light, 12-hour dark) photoperiod. Standard husbandry techniques were used [B. A. Brewer, R. C. Lacy, M. L. Foster, G. Alaks, *J. Hered.* **81**, 257 (1990)]. The diet was standard mouse chow, ad libitum. A minimum of three (and a maximum of four) generations of mice were produced in captivity before any mice were released. Pairings were chosen to produce animals with inbreeding coefficients of either 0.0 (noninbred) or 0.25 (from full-sib pairings). We assumed that wild-caught individuals were unrelated. Litters were weaned at 20 days after birth; weanlings were sexed, given a unique ear punch, weighed, and housed with same-sex, similar-age animals. All manipulations were conducted without anesthesia.
14. The mean age of all mice at release was 117 days (minimum age of 42 days, maximum age of 207 days). Inbred and noninbred mice did not differ in age in any of the three independent releases (release 1: mean age of inbreds = 152.3 days, mean age of noninbreds = 152.7 days, test for difference in ages (t) = 0.09, 274 df, P = 0.93; release 2: mean age of inbreds = 81.2 days, mean age of noninbreds = 80.4 days, t = 0.29, 255 df, P = 0.77; release 3: mean age of inbreds = 87.1 days, mean age of noninbreds = 81.5 days, t = 1.54, 160 df, P = 0.12). Releases occurred on three dates in 1991 (25 April, 19 June, and 12 August). Trapping began the day after each release and continued for three subsequent nights. Two trappings of three consecutive nights each were also conducted during the 2 weeks after each release, beginning 8 and 15 days after release. There was thus a total of three releases and 27 recapture occasions. Mice released in the first batch were subject to recapture at all 27 recapture occasions, whereas mice released in the last batch were only subject to 9 recapture occasions. Small aluminum Sherman live traps, baited either with peanut butter and oatmeal or with mouse chow, were arranged in three trap lines per site, with approximately 125 traps per site and 1 m between traps. Traps were baited late in the afternoon and checked and closed the following morning. All captured mice were brought into the lab for identification and weighing. Newly caught wild mice were ear-punched for identification. Within 45 min of capture, all mice were returned to the sites in which they had been captured.
15. The total rate at which mice were captured after release, 0.0128 animal per trap night, based on pooling captures of released mice and wild mice, was considerably lower than the rate observed when stock populations were collected for lab-rearing (0.1633). This difference in rate indicates that the population density, even after release, was lower than it had been in the natural population 2 years earlier. It is not known whether this decrease in population density was caused by disease, increased predation, or some other cause.
16. Data are available over the Internet from D. G. Gilbert, *IUBIO Archive of Molecular and General Biology Software and Data*, an Internet resource available by anonymous ftp to ftp.bio.indiana.edu (1989) in the file biol/data/peromys-rh.txt.
17. Jolly-Seber estimates are described by G. A. F. Seber, *The Estimation of Animal Abundance and Related Parameters* (Griffin, London, ed. 2 1982). Survivorship from sample time t to $t+1$ is calculated as $\phi_t = M_{t+1}/[M_t + (s_t - m_t)]$, where M_t is the estimated size of the marked population just before t, s_t is the total number of animals released at t, and m_t is the number of marked animals caught at t. The value of M_t is calculated as $m_t + [(s_t + 1)Z_t/(R_t + 1)]$, where R_t is the number of animals released at t and caught in some later sample and Z_t is the number of individuals marked before t, not caught within time t but afterward in some later sample. Jolly-Seber estimates are valid if the time intervals between samples are unequal. Estimates of ϕ_t at adjacent time intervals cannot be treated as independent estimates of survival rate, so they have been treated as repeated measures of survival for each group of individuals. Separate estimates of ϕ_t were calculated for each inbreeding group of each sex within a release batch, and these separate estimates were treated as independent replicates. Weekly survivorship estimates were calculated as the product of the three survivorship estimates within a given week. After the first week, the first survivorship estimate in each week encompassed the 4-day interval since the previous capture date. A factorial repeated-measures ANOVA was then performed on six weekly survivorship estimates of mice from the first two releases. This analysis allowed us to compare survival in inbred and noninbred individuals during the first 10 weeks after release for mice in the first two releases. Animals in the third release were subject to recapture only for 3 weeks after their release, so there were insufficient weekly survivorship estimates for this release to allow analysis by repeated-measures ANOVA (mice from the third release were included in the maximum likelihood analysis). We treated the weekly Jolly-Seber estimates from a given sex-by-inbreeding-by-release group as nonindependent repeated measures. Using inbreeding status, sex, and sex-by-inbreeding interaction as fixed effects in the ANOVA, we found a significant effect of inbreeding [$F_{(1,1)}$ = 10.80, P = 0.03] on survivorship. The sex [$F_{(1,1)}$ = 1.17, P = 0.34] and sex-by-inbreeding [$F_{(1,4)}$ = 0.32, P = 0.60] effects were not significant. When sex was removed from the model, the effect of inbreeding became even more significant [$F_{(1,6)}$ = 11.45, P = 0.01]. The time interval between release and the last recapture for each animal was also significantly shorter for inbred individuals than for noninbred individuals [pooled variances: t = 1.752, 120 df, P = 0.04 (one-tailed test)]. We found no significant trends of survivorship with time [time-by-inbreeding effect: $F_{(5,30)}$ = 0.46, P = 0.80].
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19. We tested a series of nested models that included a model in which survival and recapture probabilities were constant with respect to time and inbreeding status [ϕ, probability of recapture (p)]; a model in which both survival and recapture varied with respect to time, inbreeding status, and time-by-inbreeding interaction ($\phi_{t,r}$, $p_{t,r}$); and all models that could be nested between these two models [($\phi_{t,r}$, p_t), (ϕ_t, $p_{t,r}$), ($\phi_{t,r}$, p_t), (ϕ_t, $p_{t,r}$), (ϕ_t, p_t), (ϕ_t, p_t), (ϕ_t, p_t), (ϕ_t, p_t), (ϕ_t, p), (ϕ_t, p), (ϕ, p_t), and (ϕ, p_t)]. The fit of the data to the assumptions of the Cormak-Jolly-Seber model was tested with Burnham's tests 3 and 2 for goodness of fit of the data to the most general model ($\phi_{t,r}$, $p_{t,r}$) (*18*) (tests 2 and 3: χ^2 = 22.23, 19 df, P = 0.12). This result indicates no significant deviations from the assumptions of the model. Akaike's function optimization framework (*21*) was then used to select the best fitting model of those tested, which was the model including only an effect of inbreeding on survival and an effect of time on recapture probability, (ϕ_t, p_t). This model had the smallest value for the Akaike Information Criterion (AIC = 1057.77). The AIC of the next best–fitting model was 1059.36, for the model (ϕ, p_t) (survival is constant, and recapture rate varies with time). The difference in goodness of fit between these two best fitting models was then tested for significance by computing the likelihood ratio test for 1 df (LRT = 3.58), which is significant by a one-sided test at P = 0.03. The test is one-sided because of the a priori expectation that inbred mice have lower survivorship than noninbred mice. The AIC is used in selecting the best fitting model to eliminate the problems of statistical inference that arise if multiple tests of significance are performed [see (*18*)]. The effect of inbreeding status on the recapture probabilities of surviving mice was not significant [LRT = 12.11 for the difference between the best fitting model (ϕ_t, p_t) and the (ϕ_t, $p_{t,r}$) model, with 12 df and P = 0.56]. Both the LRT and the AIC test indicate that the effect of inbreeding on survival probabilities is statistically significant in the model.
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