Male–male competition magnifies inbreeding depression in wild house mice

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The detrimental effects of inbreeding on vertebrates are well documented for early stages of the life cycle in the laboratory. However, the consequences of inbreeding on long-term survival and reproductive success (Darwinian fitness) are uncertain for vertebrates in the wild. Here, we report direct experimental evidence for vertebrates that competition increases the harmful effects of inbreeding on offspring survival and reproduction. We compared the fitness of inbred (from full-sib matings) and outbred wild house mice (*Mus domesticus*) in large, seminatural enclosures. Inbred males sired only one-fifth as many surviving offspring as outbred males because of their poor competitive ability and survivorship. In laboratory conditions, inbreeding had relatively minor effects on male reproductive success and no effect on survivorship. Seminatural conditions did not increase inbreeding depression for females, probably because females were not competing for any critical resources. The overall reduction in fitness from inbreeding was 57%, which is 4.5 times as great as previous estimates from the laboratory. These results have important implications for medicine, conservation, evolutionary biology, and functional genomics.

Inbreeding increases the overall homozygosity of offspring compared with mating between nonkin (outbreeding) (1). Elevated homozygosity has harmful consequences (''inbreeding depression'') (2) because it unmasks the expression of recessive deleterious alleles (3, 4) generated by mutation at each generation (5, 6); it also reduces heterozygote advantage (7). Inbreeding may be detrimental to human health (8–10), agricultural productivity (11), and the persistence of small, endangered populations (12, 13); however, the magnitude and long-term importance of inbreeding depression for vertebrates in the wild are uncertain and controversial (14).

Correlative field studies in which inbreeding is inferred from pedigrees report inconsistent effects of inbreeding on fitness (15–18), and those that infer levels of inbreeding from molecular data have revealed inbreeding depression in some (19–22) but not all (23) cases. Furthermore, the vast majority of estimates of the magnitude of inbreeding depression are based on juvenile survival in artificial conditions (3, 24–26). Such captive studies are likely to underestimate the consequences of inbreeding for two reasons. First, they neglect the effects of deleterious alleles that affect individuals in the adult phase of the life cycle (9, 27). Second, they cannot reveal the effects of conditionally deleterious alleles (6, 28), whose effects will be revealed only in the presence of natural stresses, such as harsh climates, food shortages, competition, predation, and parasitism. Experimental studies of invertebrates and plants reveal that inbreeding depression may be greater when measured under stressful environments (29–31); therefore, we need to determine the effects of inbreeding for vertebrates under natural conditions.

Surprisingly, there has been no experimental measurement of the effects of inbreeding on both adult survival and reproduction for any vertebrate living in the wild or even under stressful conditions designed to mimic nature. The only experimental study of inbreeding depression for a vertebrate in natural conditions is a mark–recapture study of white-footed mice (*Peromyscus leucopus*), which reported a 44% reduction in survivorship because of inbreeding (32). Survival was estimated indirectly (trapability) and based on 123 mice recaptured and followed over 10 weeks. Inbreeding was not correlated with ''survivorship'' among the 663 mice that were never recaptured. Furthermore, neither long-term survival nor reproductive success was measured, leaving open the possibility that enhanced reproductive success by inbred individuals outweighs reduced survivorship (14, 18).

To obtain a more realistic estimate of the fitness costs of inbreeding under natural conditions, we measured the relative fitness of inbred and outbred adult house mice (*Mus domesticus*) living in competitive, seminatural population enclosures (33). We trapped wild mice and mated their progeny to produce offspring, which were inbred (inbreeding coefficient, $F_i = 0.25$) or outbred ($F_i = 0.00$) (Fig. 1). We simultaneously released 144 of these F_2 mice into six replicate enclosures (24 mice per enclosure), where males fight for territories and compete for females. We used sex-specific genetic markers to measure the reproductive success of inbred and outbred mice over a 10-month period. We measured survivorship and male success in agonistic interactions as possible determinants of reproductive success. During the term of the enclosure populations, we also measured the survival of 700 F_2 mice and the reproductive success of 220 F_2 mice housed in standard laboratory conditions (i.e., noncompetitive conditions).

Materials and Methods

Mice and Colony Conditions. One hundred and fifty-seven wild house mice were trapped from two locations (10 km apart) near Gainesville, FL. The breeding design used to produce inbred and outbred mice is shown in Fig. 1. Mice were housed in our colony (laboratory conditions) in 13 × 18 × 29-cm clear acrylic mouse cages and provided with pine bedding, cotton nesting material, and food and water *ad libitum*. Animal rooms were maintained at 22 ± 2°C, with a light/dark cycle adjusted monthly to mimic natural day lengths. Breeding pairs were housed in opaque cages and provided with apples and sunflower seeds as dietary supplements. Males were removed at 18 days postpairing, and females were checked for litters every day thereafter. Litters were counted on the day of birth and left undisturbed for 21 days, then weaned, removed from the mother, and housed with same-sex sibs.

Seminatural Enclosures. House mice are commensal and live in human-built structures. We released populations of mice into seminatural enclosures designed to mimic their natural habitat and social environment, yet still allow observation of behaviors important to reproductive success (below). Six 4.9 × 9.8-m

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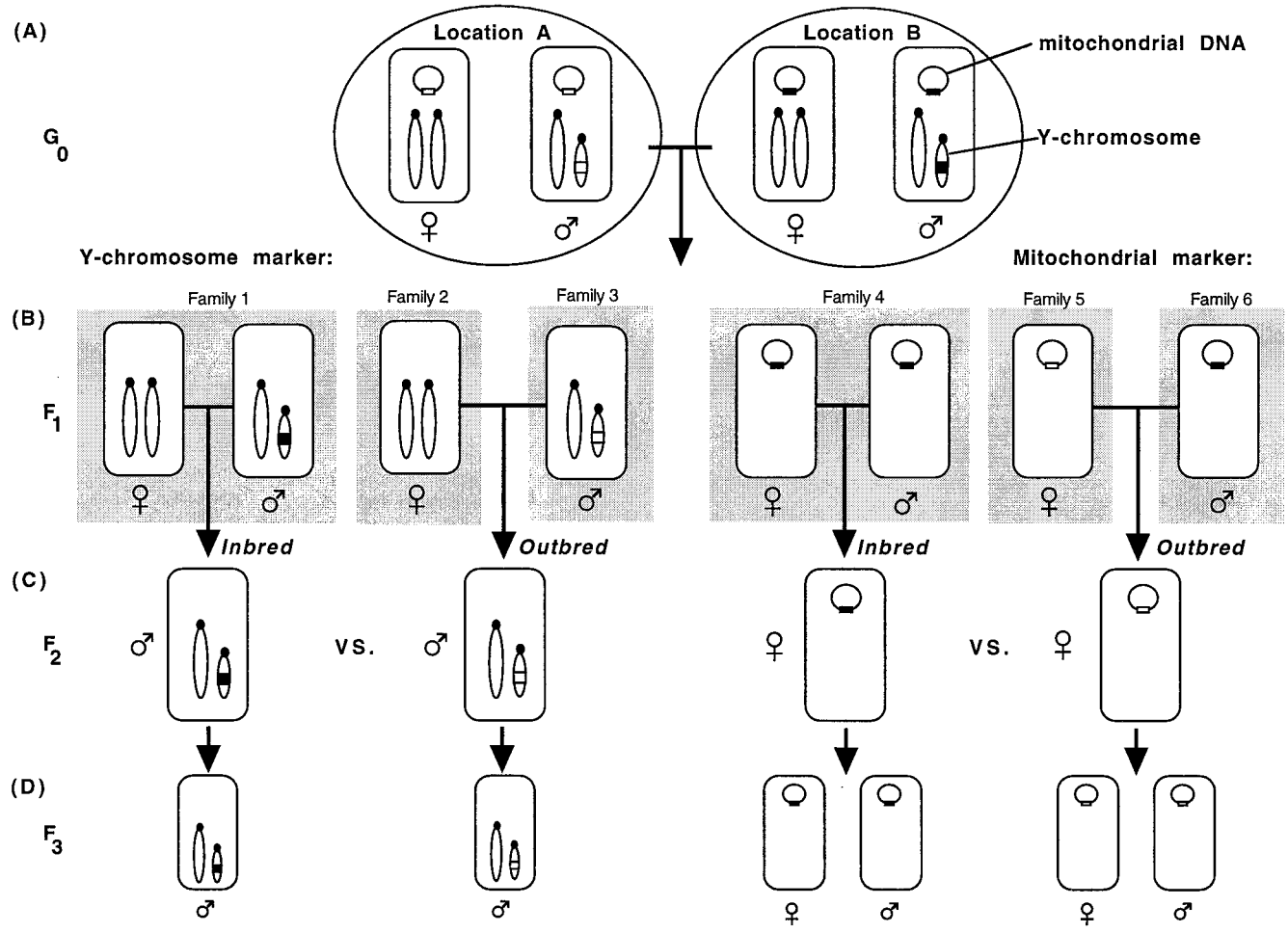

Fig. 1. The breeding design. (*A*) Wild house mice from two locations were intercrossed to ensure that no matings occurred between relatives (i.e., no inbred F_1 mice). The two populations displayed nearly fixed differences for genetic markers (open and closed symbols) on the Y chromosome and mtDNA. (*B*) F_1 mice were mated either within families (siblings) to produce inbred offspring ($F_i = 0.25$; e.g., Family 1) or between families (unrelated mice) to produce outbred mice ($F_i = 0.00$; e.g., Families 2 and 3). (*C*) The inbred and outbred F_2 mice were introduced into population enclosures or housed in the laboratory. Inbred and outbred population founders had distinct Y chromosome and mtDNA markers so that their progeny could be distinguished. (*D*) The F_3 progeny born in the enclosures were typed genetically to determine the relative reproductive success of inbred and outbred males and females.

enclosures are housed within a 320-m^2 heated, predator-proof concrete and steel building. Each enclosure is subdivided into eight equal subsections by 46-cm-high hardware cloth (1.25-cm grids), and each subsection contains an additional spiral of hardware cloth. The screening provides environmental complexity important for normal behavior in house mice (33), and males tend to use the partitions as territorial boundaries. Food, water, nest boxes, nesting material, and wood chips were available *ad libitum* in each subsection. We also included four "refuges" per enclosure (nest boxes hanging 1 m above the ground, which mice could reach only by climbing a wire), which allowed subordinate males and females to escape harassment by dominant males. Some parasites may have been lost in colony conditions, but we made no attempt to reduce parasite loads, as indicated by a 92% prevalence (101/110) of two nematodes (*Syphacia obvelata* and *Aspicularis tetraptera*) in founder mice.

Enclosure Populations. The 144 enclosure mice were the F_2 descendants of 84 wild-trapped mice (21 males and 21 females from each of two Florida trapping locations). Each of six replicate populations consisted of 24 mice per enclosure (eight males and 16 females), which is within the range of typical densities of wild house mice (34). In each enclosure, half of the mice were inbred and half were outbred. Founders for each population were chosen randomly except for the following constraints: within each enclosure, inbred males and females had distinct Y chromosome and mtDNA markers relative to the outbred males and females in the population (see below). Mice within a population enclosure were age-matched (mean $\pm$ SE = 144 $\pm$ 5 days), and they were chosen to minimize relatedness of individuals within populations. Within an enclosure, males had no relatives and females had no siblings. Females did share enclosures with unfamiliar cousins (1–4 cousin pairs per pen), but these cousin pairs were balanced across inbreeding levels (i.e., the cousin of an inbred female was an outbred female). This design reduced the possibility that kin-based mating behaviors would influence the results.

Behavioral Observations. Enclosure mice were marked with unique ear punches for individual identification by using modified, close-focus binoculars. Agonistic interactions were recorded by observers, who were unaware of the inbreeding status of the mice, for 1 h per enclosure per night, for 3–5 nights per week, throughout the experiment (total = 492 observer h; mean = 82 observer h per enclosure). Behavioral data were collected at dusk during peak activity by two observers, so that

both mice could be positively identified during dyadic interactions. "Percent wins" for each male was calculated as (number of chases + attacks)/(chases + attacks + flights). Males were considered "territorial" if they won most agonistic interactions within at least one subsection of the enclosure (>80% was arbitrarily chosen); otherwise they were considered "subordinate." Censuses to monitor adult survival were taken daily, and nest checks were conducted every 10 days to obtain information on nest locations, birth of litters, and loss of pups. When enclosure-born pups reached weaning age (21 days), they were captured and ear-punched for identification. Ear-punch tissue was used for DNA extraction and subsequent genetic typing. These mice were released back into the enclosures and then removed before reaching sexual maturity at 45 days.

Molecular Markers. We determined the reproductive success of inbred and outbred mice in the enclosures by taking advantage of polymorphisms segregating in the wild-trapped populations. To distinguish whether male parents were inbred or outbred, we developed PCR primers (5′-CAGGGTTTCTCTCTAGCACA and 5′-CACAACTGGGCTTTGCACATTG) for a microsatellite marker in the inverted repeat region near *Sry* on the Y chromosome (35). To determine female parentage we used a length variant in the control region of the mitochondrial genome (5′-TTGGTTTCACGGAGGATGGT and 5′-CACCACCAGCACCCAAAGCT). For each population, founders were chosen such that inbred and outbred individuals possessed nonoverlapping alleles for these two sex-specific markers. We did not determine exact parentage for the pups, but these markers enabled us to determine whether the mother was inbred or outbred (for all pups) and whether the father was inbred or outbred for the sons. Using only sons to evaluate male reproductive success might be misleading if inbred and outbred males produced different sex ratios; however, we found no differences in the sex ratios from inbred and outbred males in our laboratory (*t* test, $n = 55$ litters, $t = 0.45$, $P = 0.38$). The genetic markers characterizing inbred and outbred mice were reversed in half of the populations to avoid confounding the effects of inbreeding with effects caused by marker alleles or closely linked genes.

Analyses. Fitness components from the early phase of the life cycle were measured in our laboratory. Litter size was measured for 188 litters from 112 breeding pairs (58 unrelated pairs that produced "outbred" litters and 54 brother–sister pairs that produced "inbred" litters). We analyzed the effect of inbreeding on litter size with multiple linear regression (MLR) and included the litter's inbreeding coefficient (outbred $F_i = 0.00$; inbred $F_i = 0.25$), mother's weight, and parity (first or second litter) in the model. The masses of 1,069 pups at weaning (21 days) and 1,055 of these mice at adulthood (50 days) were examined with an MLR model that included the pup's inbreeding coefficient, sex, and number weaned from its litter.

The fitness of adult mice was measured in both the enclosures and the laboratory. The reproductive success of inbred and outbred mice was compared by using contingency and goodness-of-fit χ^2 tests on the number of offspring. "Total offspring" included dead offspring collected throughout the course of the experiment and *in utero* offspring from pregnant females at the end of the experiment. "Weaned offspring" was calculated as the number of pups that reached weaning age (21 days or older) plus preweaned offspring alive at the end of the experiment. For the enclosures, we report a conservative test, using enclosure populations as independent replicates, and a liberal test, using data for all populations combined. The results of both tests are consistent. Reproductive success in the laboratory was based on first litters of 110 breeding pairs in which inbred (I) and outbred (O) males and females were paired in all possible inbreeding × sex combinations (26 O × O, 29 O × I, 26 I × O, and 29 I × I crosses). Laboratory breeding pairs were from the same cohort as the enclosure mice and were allowed 50 days to produce a litter.

Adult survivorship of 144 enclosure mice and 700 mice from the same cohort maintained in the laboratory was analyzed by using Kaplan–Meier analyses (Wilcoxon *P* values are shown). Fisher exact tests were used to analyze the effects of inbreeding on social status among males in the enclosures. One-sided tests were used because inbreeding is known to impair male dominance and territorial success (36, 37). Statistical analyses were performed by using JMP 3.1 (SAS Institute, Cary, NC) and INSTAT 2.01 (GraphPad, San Diego).

Results and Discussion

Prereproductive Fitness. In the laboratory, inbreeding decreased litter size by 11% (mean ± SE of outbred litters = 6.83 ± 0.24, inbred litters = 6.09 ± 0.25; $F_{1,182} = 5.93$, $P = 0.016$). Despite coming from smaller litters, inbred pups were significantly smaller at weaning (outbred male mass = 7.69 ± 0.07 g, inbred male mass = 7.39 ± 0.07 g; outbred female mass = 7.42 ± 0.07 g, inbred female mass = 7.16 ± 0.07 g; inbreeding $F_{1,1065} = 19.04$, $P < 0.0001$; sex $F_{1,1065} = 13.49$, $P < 0.001$). At adulthood, the size difference between inbred and outbred animals was no longer significant (outbred male mass = 15.49 ± 0.12 g, inbred male mass = 15.03 ± 0.14 g; outbred female mass = 12.57 ± 0.10 g, inbred female mass = 12.47 ± 0.11 g; inbreeding $F_{1,1051} = 3.69$, $P = 0.055$; sex $F_{1,1051} = 11.25$, $P < 0.001$). There was no size difference between inbred and outbred male founders when the enclosure populations were begun (outbred male mass = 17.29 ± 0.54 g, inbred male mass = 17.60 ± 0.54 g; $t = 0.41$, df = 46, $P = 0.68$).

Reproductive Success. Adult inbred males sired fewer total offspring than outbred males in both the enclosures and the laboratory (Fig. 2 *A* and *B*). In the enclosures, inbred males sired significantly fewer offspring in all six populations (*P* always < 0.05; Sign test, $P = 0.016$), and they had only 28% as many total offspring as outbred males overall. Furthermore, the offspring of inbred males had lower survivorship than those of outbred males (pup survivorship was 64% for inbred vs. 75% for outbred, $n = 713$, $\chi^2 = 6.3$, $P < 0.012$). Consequently, inbred males had only 19% as many pups as outbred males that survived to weaning age. The disparity in reproductive success between inbred and outbred males increased over time for both the total number of offspring (not shown) and the number of weaned offspring (Fig. 3).

In the laboratory, inbred males had 64% as many weaned offspring as outbred males. This was due to a reduction in the number of pups born because the survivorship of the progeny of inbred males was not significantly affected (84% for offspring of inbred males and 89% for offspring from outbred males; $n = 303$, $\chi^2 = 1.3$, $P = 0.13$). The fitness declines for males in the enclosure populations were significantly greater than in the laboratory (total offspring, $n = 817$, $\chi^2 = 32.2$, $P < 0.0001$, weaned offspring, $n = 501$, $\chi^2 = 32.2$, $P < 0.001$). Thus, inbred males had lower reproductive success than outbred males, and this difference was magnified in the competitive conditions of the enclosures. Inbreeding impairs mammalian male reproductive mechanisms such as ejaculate quality (38–40) and paternal care (27). Inbreeding decreases male fertility in quail (41), and our data indicate that it can also reduce male reproductive success in a mammal.

Inbreeding also significantly decreased the reproductive success of females in both the enclosures and the laboratory (Fig. 2 *C* and *D*). In the enclosures, inbred females had fewer offspring in five of six and significantly fewer offspring ($P < 0.05$) in three of six populations. Overall, inbred females in the enclosures produced significantly fewer offspring than outbred females

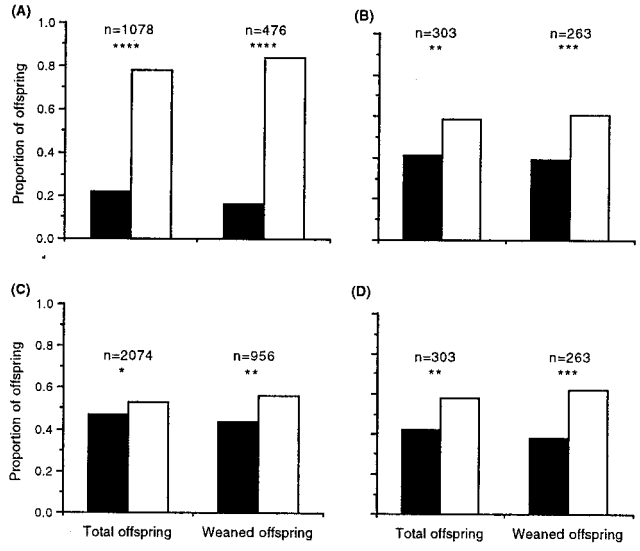

Fig. 2. The reproductive success of inbred (solid bars) and outbred (open bars) mice. (*A*) Males in enclosures. Inbred males had significantly lower reproductive success than outbred males (total offspring, $n = 1028$, $\chi^2 = 87.7$, $P < 0.0001$; weaned offspring, $n = 476$, $\chi^2 = 60.8$, $P < 0.0001$). (*B*) Males in laboratory. The reproductive success of inbred males was significantly lower than outbred males (total offspring, $n = 606$, $\chi^2 = 10.35$, $P < 0.005$; weaned offspring, $n = 526$, $\chi^2 = 11.9$, $P < 0.001$). (*C*) Females in enclosures. Inbred females had significantly lower reproductive success than outbred females (total offspring, $n = 2074$, $\chi^2 = 4.7$, $P < 0.05$; weaned offspring, $n = 956$, $\chi^2 = 7.3$, $P < 0.01$). (*D*) Females in laboratory. The reproductive success of inbred females was significantly lower than outbred females (total offspring, $n = 606$, $\chi^2 = 6.9$, $P < 0.01$; weaned offspring, $n = 526$, $\chi^2 = 16$, $P < 0.001$) (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

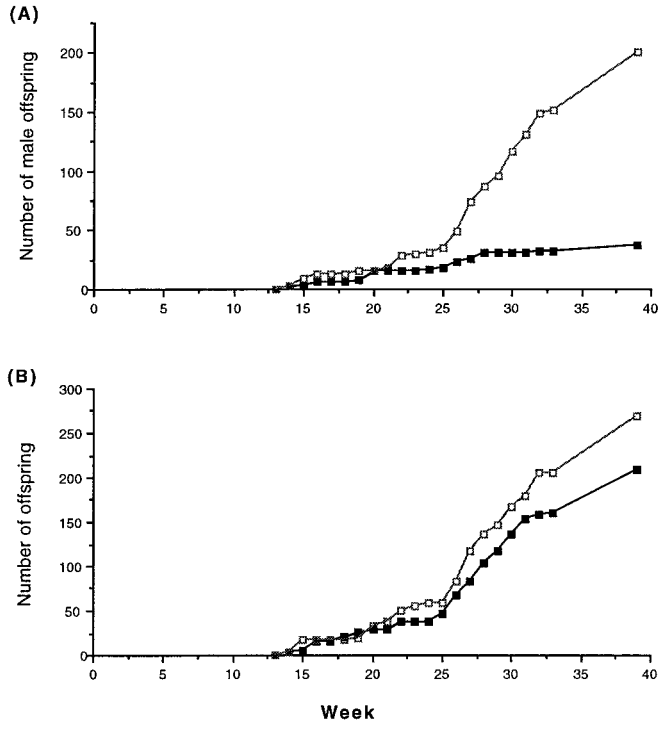

Fig. 3. Cumulative number of weaned offspring from inbred (■) and outbred (□) mice in the enclosures. (*A*) Males. Outbred males produced twice as many young as inbred males during the first 8 weeks of pup production and eight times as many during the last 8 weeks of the experiment (8-week time periods chosen arbitrarily) ($n = 265$, $\chi^2 = 14.7$, $P < 0.001$). (*B*) Females. There was no significant difference in the reproductive success of inbred and outbred females during the first 8 weeks; however, outbred females produced 1.5 times as many offspring as inbred females during the last 8 weeks of the experiment ($n = 521$, $\chi^2 = 5.2$, $P < 0.025$). Weekly nest checks were stopped from weeks 33 to 38.

(only 87% as many total offspring), and their offspring had lower survivorship (pup survivorship was 68% for inbred vs. 73% for outbred, $n = 1460$, $\chi^2 = 4.3$, $P < 0.05$). As a result, inbred females produced only 78% as many weaned offspring as outbred females. The fitness decline because of inbreeding for females increased over time for the number of weaned offspring (Fig. 3), but not for the number of total offspring (data not shown). Thus, inbreeding significantly depressed both mating and rearing success of males, but only pup rearing of females. In the laboratory, inbred females produced 60% as many surviving offspring as outbred females (Fig. 2 *C* and *D*), which was not significantly different from the fitness decline for females in enclosures (total offspring, $n = 1340$, $\chi^2 = 1.6$, $P = 0.21$; weaned offspring, $n = 741$, $\chi^2 = 2.3$, $P = 0.13$).

Male Competitive Ability. The low reproductive success of inbred males can be explained partly by their inability to obtain territories. Territories are critical for male reproduction because females mate almost exclusively with dominant, territorial males (42). Significantly fewer inbred males became territorial than outbred males (10/24 inbred vs. 17/24 outbred males; Fisher exact test, $P = 0.04$). This is consistent with other evidence that inbreeding decreases a male's success in aggressive encounters (36) and his ability to obtain territories (37). Furthermore, when inbred males acquired territories, they resided in sections having both fewer resident females (Wilcoxon test signed rank, $P = 0.03$) and fewer pups born ($P = 0.03$). This suggests that inbred males obtained suboptimal territories or that females prefer to nest in the territories of outbred males.

Adult Survivorship. The low fitness of inbred males in the enclosures was due in part to their reduced survivorship (Fig. 4). Males in the enclosures suffered high mortality (25/48 males died), and 68% (17/25) of the males that died were inbred. Moreover, among territorial males, inbred males displayed significantly lower survivorship (9/10 inbred and 3/17 outbred, territorial males died). Survivorship was significantly lower for males than females (only 6/96 females died; Kaplan–Meier, $P = 0.0001$), probably because males engaged in significantly more aggressive (injurious) interactions than females (Wilcoxon rank sum, $P < 0.0001$). Inbreeding had no significant effect on survivorship of females in the enclosures (three inbred and three outbred females died); therefore, survivorship cannot explain the low reproduction of inbred females. In the laboratory, survivorship rates did not differ between inbred and outbred males (Kaplan–Meier, $P = 0.99$) nor between females (Kaplan–Meier, $P = 0.18$). These data indicate that inbred males have reduced survivorship in competitive conditions and have difficulty paying the high costs of defending territories (43). The effects of inbreeding on adult survival are rarely examined in laboratory conditions, but recent studies of wild populations have revealed inbreeding depression of adult survival for male and female white-footed mice (32), sheep (22), and song sparrows (44).

Summary. Our results indicate that the fitness consequences of inbreeding are much greater than previous estimates for house mice (24, 25) and are consistent with the severe inbreeding depression of fitness displayed by *Drosophila* living in competitive conditions (45). First, inbreeding caused an 11% reduction in litter size in the laboratory, which is comparable to previous

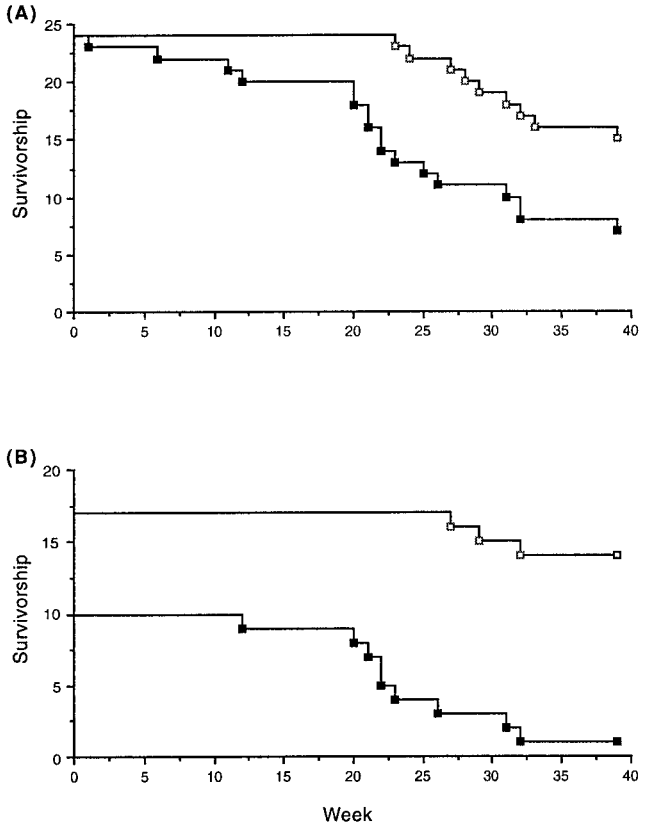

Fig. 4. Survivorship of inbred (■) and outbred (□) males in the enclosures over time. (*A*) All males. Inbred males had significantly lower rates of survivorship than outbred males (the first 11 males to die were all inbred) (Kaplan–Meier, $P = 0.0012$). (*B*) Territorial males. Inbred males had significantly lower rates of survivorship than outbred males (Kaplan–Meier, $P = 0.0001$). The number of outbred and inbred territorial males differed initially (see text).

studies of house mice (24, 25). Inbreeding had no effect on birth-to-weaning survival. Second, inbreeding resulted in a 52% mean fitness decline for adult mice in seminatural enclosures (81% for males and 22% for females). Taken together, the overall fitness reduction ($1 - w$, where w = fitness) because of one generation of full-sib mating is $1 - \{0.89 \times [(0.19 + 0.78)/2]\} = 57\%$. This fitness decline is 4.5 times as great as estimates from previous studies based on juvenile survival in laboratory conditions (24, 25) and even greater than the 40% fitness decline observed in those studies after six generations of full-sib matings ($F_i = 0.73$). These results cannot be attributed to unusually large mutational loads in our wild mouse populations because our laboratory results for juvenile survival are comparable to other studies (24, 25).

We attribute our higher estimate of inbreeding depression to two major differences between this study and earlier ones. First, we measured the consequences of inbreeding over a larger part of the life cycle than previous studies of vertebrates. This is important because fitness differences accumulate throughout individuals' lifetimes (46), and late-acting deleterious alleles are less likely to be removed by natural selection than early-acting mutations (47). We found evidence that inbreeding depression is greater in later life, which also has been shown for *Drosophila* (48) and some plants (49).

Second, our study allowed male–male competition, which greatly magnified inbreeding depression of male reproductive success (i.e., in the noncompetitive laboratory, inbreeding caused a relatively small fitness decline, Fig. 2*B*). Inbred males had reduced reproductive success because they were poor at obtaining territories and surviving during the defense of territories. Without exact parentage data, we cannot estimate the relative contribution that reduced fecundity and viability made to the total fitness decline. Male-biased inbreeding depression also has been reported for invertebrates housed in competitive, social conditions (50–52). However, decreased reproductive success from inbreeding depression is female-biased in wild song sparrows (44). Thus, the sex whose fitness is decreased most by inbreeding may vary according to the behavioral ecology of the species under study. The male bias we observed might have been exaggerated in our populations because the resources over which female mice compete (food, water, and nesting sites) were not limiting.

Our fitness estimates of inbreeding depression are likely to be conservative underestimates for several reasons. Inbred individuals may be differentially susceptible to other factors that our enclosures excluded, such as predation, a broad spectrum of infectious diseases, starvation, and temperature extremes (22, 32, 44). Furthermore, our enclosures did not allow dispersal, and because inbred males are poor competitors, they may tend to disperse, increasing their risk of starvation and predation (23). Finally, we measured the quantity of offspring produced by inbred and outbred parents, not their quality. If inbred parents provide poor parental care, then their progeny will be lower in quality than those from outbred parents [although the opposite has been suggested (14)], and they will have fewer children and grandchildren than outbred parents in the wild. Thus, it is unlikely that our study artificially inflated the effect of inbreeding on fitness.

Implications. This study provides experimental evidence for vertebrates that inbreeding depression on both adult survival and reproduction is magnified under competitive conditions, which suggests that inbreeding in the wild can be extremely severe (15, 29, 32, 44). Thus, these data suggest that the recessive mutational load is greater than is often assumed (8), which has important implications for several problems in biology.

First, the negative consequences of inbreeding on human health are well documented, but their quantitative effects are controversial (8). We suggest that inbreeding depression in humans may be grossly underestimated because studies usually are based solely on juvenile survival. In our study, inbreeding had its largest fitness effect during the adult phase of the life cycle, and a considerable portion of this effect was due to reduced competitive ability. Therefore, if the effect of inbreeding on human adults were measured in terms of health and vigor, it might reveal substantial reductions in the quality of human life. This prediction is consistent with recent reports that inbreeding is associated with decreased fertility (9) and increased cancer risk (10) in humans.

Second, it has been debated whether inbreeding depression will interfere with endangered species conservation (53, 54). Our estimate of inbreeding depression depended on whether it was based on absolute inviability or relative competitive ability [i.e., "hard" vs. "soft" selection (55)]. Our study revealed very strong soft selection—the fitness of inbred males was severely reduced in competition with outbred males. Because most animals in nature must cope with predators, parasites, and competitors, the inability to cope with biotic stresses seen here may translate to hard selection, which can result in reduced population growth and extinction (12). Furthermore, the vigor of animals in captivity may not predict their vigor in the wild (32). Thus, captive-breeding and reintroduction programs will benefit from the production of noninbred individuals better able to cope with environmental insults (29).

Third, the importance of inbreeding avoidance behaviors has been controversial because of the uncertainty over the fitness

consequences of inbreeding depression in the wild (56). Our results suggest that there will be strong selection favoring mechanisms to avoid mating with close kin. They also suggest that the fitness consequences for mating with distantly related individuals, such as cousins, may be worse than usually assumed (44). If this is generally true, then natural selection should favor the evolution of genetic kin recognition mechanisms, which allow individuals to ''recognize'' genetic similarity and avoid mating with unfamiliar kin. For example, disassortative mating preferences based on the highly polymorphic genes of the MHC may function to reduce inbreeding (57, 58).

Finally, many studies in functional genomics have disrupted genes thought to have critical functions only to find minor or no phenotypic effects (59). These surprising results often are interpreted as functional redundancy in the genome. However, competition studies in yeast (60) and fruit flies (2, 5) indicate that harmful consequences of deleterious genes become more evident under stressful, seminatural conditions. Our results provide further evidence that competition experiments can reveal negative consequences of defective genes that go undetected in benign laboratory conditions. Thus, gene disruption studies will benefit from analyzing behavior and reproductive success of animals under more natural conditions.

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