

# EFFECTS OF INBREEDING ON REPRODUCTION AND SEX RATIO OF *RATTUS VILLOSISSIMUS*

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A retrospective study of the breeding records of a laboratory colony of an Australian rodent, *Rattus villosissimus*, demonstrated significant inbreeding depression in the number of young born per litter, with a 3.4% decrease for each 10% increase in the inbreeding coefficient. This inbreeding depression showed no evidence of amelioration as the laboratory population became highly inbred over 25 generations. Inbreeding had no effect on survival or growth rate of juveniles. There was a significant relationship between the number of young and the sex ratio of a litter, with larger litters having a higher proportion of females. Inbreeding, while decreasing the number of young per litter, did not result in a significant shift in the sex ratio.

Key words: *Rattus*, inbreeding depression, reproduction, viability, sex allocation, life-history strategies

Inbreeding has been known to cause a variety of deleterious effects in many species for more than a century (Wright, 1977). Various theories have been proposed to explain the preponderance of inbreeding depression, and yet experimental data allowing discrimination between hypotheses are scant and difficult to obtain (Charlesworth and Charlesworth, 1987). The hypothesized causes of inbreeding depression fall into two categories; the reduction of fitness in inbred organisms due to homozygosity of individually rare, deleterious, recessive alleles, and general heterosis, a fitness advantage of increased heterozygosity across the genome (Crow, 1952; Lerner, 1954; Mitton, 1993).

In this study, we report a retrospective analysis of the effect of inbreeding on reproductive performance in a laboratory colony of Australian rats, *Rattus villosissimus*. The breeding colony was not maintained originally for the purpose of studying reproductive performance, so some data (e.g., body mass) are available only from some

litters. The complete pedigree of the colony was recorded, however, and substantial information on the effects of inbreeding are available in a pedigree of >1,400 laboratory-reared rats that descended from eight animals imported from Australia. The ancestry of most of the pedigree traces to three founders, and the colony became considerably inbred (inbreeding coefficients up to  $F = 0.80$ ) over 25 generations in the laboratory.

In the examination of breeding records for this colony of *R. villosissimus*, we noted complex, but informative, relationships among the number of offspring per litter, mass of offspring, the sex ratio of the litter, and inbreeding. The ability and flexibility of mammals to adjust sex ratios of litters adaptively has been a topic of some debate (Charnov, 1982; Clutton-Brock and Albon, 1982; Williams, 1979). McClure (1981) showed that litters of woodrats, *Neotoma floridana*, become increasingly female-biased due to greater mortality of male infants when mothers are stressed by restricted

food. Moreover, the deaths of male offspring seemed to be due to active discrimination against them by their food-stressed mothers. Verme (1965, 1969) and others (Clutton-Brock and Albon, 1982) found a shift toward male-biased sex ratios in deer (*Odocoileus virginianus*) that were nutritionally stressed. Inbreeding can be a stress, and it often causes reduced litter size, smaller birth weights and weaning weights, and greater mortality of juveniles (Ralls et al., 1988; Wright, 1977). Inbreeding depression can cause differential survival between males and females (Lacy et al., 1993). It is, therefore, of interest to determine the extent to which the sex ratios of litters might be affected or adjusted in response to inbreeding.

Although there are extensive data on the effects of inbreeding on laboratory strains of rodents and on domesticated livestock, relatively few studies have examined the effects of inbreeding in non-domesticated, experimental populations (Lacy et al., 1993). It is difficult to generalize the magnitude of inbreeding depression observed in domesticated and laboratory strains that have been subjected to long-term artificial selection to the effects expected in stocks recently derived from wild populations (Lacy, 1993). There is no certainty that the underlying genetic basis responsible for inbreeding depression would be the same in gene pools of natural populations as it would be in the highly artificial gene pools of domesticated stocks. Therefore, data on the effects of inbreeding on a diversity of recently established experimental populations are needed to provide a better understanding of the likely effects of inbreeding on natural populations.

#### MATERIALS AND METHODS

The progenitors of the *R. villosissimus* included in this study were trapped in April 1969 by J. L. Carstairs at Brunette Downs, Northern Territory, Australia, as part of a colony being established at the Division of Wildlife Research, Commonwealth Science and Industrial Research

Organizations, Canberra, Australian Capital Territory. A colony at Smith College, Northampton, Massachusetts, was derived from a pair of these wild-caught animals (sent from Australia in February 1970) plus three females and three males (sent from Australia in July 1971). The two field-caught founders of the colony were assumed to be unrelated. The second shipment represented animals captive-born in Australia in November 1970. These six animals were described as being held for a small number of generations in captivity, were likely siblings, and were supposedly unrelated to the first two rats (J. Calaby and K. Myers, pers. comm.). Of the six rats received in the second importation, however, five produced few litters and had no second-generation descendants. Thus, most of the colony descended from three imported founders.

The colony at Smith College, maintained primarily for observational purposes associated with teaching, persisted until November 1985. During its 15-year history, the colony was housed in standard caging in the animal quarters of the college, and all animals were provided with essentially the same dietary and environmental regimes. Rats were ear-punched and weighed, usually at the time of weaning, and records were kept of all pairings producing weaned progeny. Standard procedures called for weighing, measuring, and freezing animals at the time of death.

The inbreeding coefficient ( $F$ ) was calculated for each animal in the lab colony. Because there is always the possibility that some of the wild-caught animals could have been inbred or related (through recent common ancestry in the wild), the inbreeding coefficients calculated can only be verified to represent minimum estimates of the extent of inbreeding. The six founder animals in the second shipment were likely siblings, but their relationship could not be confirmed through records. Therefore, inbreeding coefficients were calculated both with the assumption that these animals were full-siblings and with the assumption that they were unrelated. Because only one of the six possible siblings contributed more than a few descendants to the colony at Smith College, the alternative assumptions about the relationships among the founders affected the inbreeding coefficients calculated for only two of the 294 litters included in the study. Trends in the data were not affected by which assumption was used to calculate inbreed-

ing in the two litters. The calculations presented were those assuming that the six rats of the second importation were siblings.

The following data on reproductive performance were obtained for each litter from the records for the colony: number and sex of young born; number and sex of young weaned; viability of offspring (number weaned/number born); mean mass of female offspring measured at or near the time of weaning; mean mass of male offspring measured at or near the time of weaning; total mass of litter; sex ratio of the litter at the time of weaning. Not all offspring were weighed, and weights were not taken at a fixed age. Therefore, mean mass of females and mean mass of males were not always based on measurement of all individuals in the litter. When only some rats within a litter were weighed, total mass of litter was estimated by adding the product of the number of males weaned and the mean mass of males that were weighed and the product of the number of females weaned and the mean mass of females weighed. In a few cases, in which only one sex was weighed but the litter contained offspring of both sexes, the litter mass could not be estimated. The number weaned, the viability, and the sex ratio at weaning could not be determined for three litters that were sacrificed prior to weaning for studies of early development.

Because offspring were weighed at various ages, the effect of age on mass was removed prior to assessment of the effect of inbreeding. Data on mass at various ages, taken primarily at weaning (21 days) and again when animals were sacrificed (usually as adults), were used to estimate the best-fit growth curves for females and for males. Gompertz, Brody, logistic, von Bertalanffy, and log-linear growth curves (Fitzhugh, 1976) were fitted, via nonlinear estimation minimizing least-square deviations (Zullinger et al., 1984), to 851 measurements of mass of males and 936 measurements of mass of females. The Gompertz curve provided the closest fit to the data, and data on mass from rats within intervals of inbreeding ( $F < 0.25$ ;  $0.25 \leq F < 0.50$ , and  $F \geq 0.50$ ) yielded Gompertz curves that were statistically indistinguishable in shape. Therefore, testing of the effects of inbreeding was conducted on deviations of log (mass) from the values predicted from the sex-specific Gompertz growth curves. The effect of inbreeding on litter mass was assessed from the deviations of log

(litter mass) from the log (predicted mass), with the predicted mass at each age determined as the mean number of females weaned times the mass predicted for females from the Gompertz curve for females plus the mean number of males weaned times the mass predicted from the Gompertz curve for males. To analyze mass during the period of most-rapid growth, we restricted analyses of the effects of inbreeding to those mass measurements taken at 21–90 days of age. Some litters were weighed on several occasions, but the inclusion of such multiple measurements in statistical analysis would violate assumptions of independence of data points. Therefore, for statistical tests we used data on mass from only the first date of measurement at or after weaning for each litter. The Gompertz curves used to calculate deviations from mean age-specific mass were estimated using all available data on mass and included some litters that were measured as young as the day of birth.

Distributions of non-transformed data deviated strongly from normality. The distributions of numbers of rats born and number weaned were both skewed to the right. Square-root transformations removed skewness and were used for the statistical tests. The viability of offspring (number weaned/number born) was skewed strongly to the left and was leptokurtic. The arcsin square-root transformation reduced both deviations from normality, but after transformation, the distribution of viability remained skewed to the left ( $g1 = -1.04$ ,  $n = 241$ ;  $P < 0.01$ ), because of a large number of litters that had 100% survival. The distribution of sex ratio of litters approximated a normal distribution with a mean ( $51.0\% \pm 1.5\% SE$ ) that was not significantly different from 50% females. The distribution of deviations from the log (mass) predicted from the Gompertz growth curves for females was not significantly different from a normal distribution. Deviations from the Gompertz curve in log (male mass) were leptokurtic ( $g2 = 1.74$ ;  $P < 0.01$ ) and skewed to the left ( $g1 = -0.93$ ,  $n = 160$ ;  $P < 0.01$ ), because a few males were unusually small. Deviations from the predicted values of log (litter mass) also were skewed moderately to the left ( $g1 = -0.78$ ,  $n = 173$ ;  $P < 0.01$ ), because a few litters were unusually small for their ages. The variances of all transformed variables were homogeneous across levels of inbreeding.

Least-squares linear regressions were used to

test the relationships between the level of inbreeding ( $F$ ) and six measures of reproductive performance: the number of young born (square root transformed); viability (arcsin square root); mass of males (deviation of log (mass) from the Gompertz growth curve); mass of females (deviation of log (mass) from the Gompertz curve); total mass of litter (deviation of log (mass) from the value predicted from the mean number of young and the sex-specific Gompertz curves); sex ratio of litter. The effects of the parity and age of the mother also were examined. Neither parity nor the age of the mother had a significant effect on any of the measures of reproductive performance, and these covariates were excluded from the regression models. In all the statistical tests, each litter was considered to be an independent data point. Therefore, the mean mass of same-sex offspring, rather than masses of individual young, was analyzed statistically. Littermates share a common rearing environment that would violate the statistical assumption of independent data points necessary to allow valid tests of mass of individual offspring.

## RESULTS

Because of the structure of the pedigree, with the population starting from just a few founder animals and becoming increasingly inbred in later generations, there was a high correlation ( $r = 0.87$ ) between the inbreeding coefficient of the female and the inbreeding coefficient of the litter. As a result, it is not possible from these data to determine whether the effect of inbreeding on characteristics of litters is due to a direct effect of inbreeding in the progeny themselves, or due to an indirect maternal effect, in which inbred females provide less-optimal maternal care, or due to the effects of inbreeding at both levels. Models assessing the effect of inbreeding of litters explained about the same amount of variance in the measures of reproductive performance as did models of the effect of inbreeding of the mother. Factoring out either inbreeding of females or inbreeding of litters left no residual associations of variables with inbreeding at the other level.

When adjusted for inbreeding, the mean number of young born per non-inbred litter

( $F = 0$  intercept from the regression of the litter size against inbreeding coefficient:  $6.46 \pm 0.37 SE$ ) and the number weaned ( $5.30 \pm 0.37 SE$ ) were similar to, but slightly below, the comparable values ( $7.1 \pm 0.3 SE$  and  $6.3 \pm 0.4 SE$ ) reported by Carstairs (1976) for laboratory-born *R. villosissimus* descended from the same collections of wild rats as were the subjects of this study. We found inbreeding to have a strong effect on the number of young born in a litter, with a 34% reduction predicted from the model for non-inbred litters to that extrapolated for fully inbred ( $F = 1.0$ ) litters (Table 1). The reduction (29%) in the number of young weaned paralleled the reduction in the number born, as inbreeding had a non-significant effect on survival of litters.

Because litters were weighed at different ages, the measures of mass of offspring and litters had to be adjusted for the growth rate before the effect of inbreeding could be analyzed. Although only a few litters were measured prior to weaning, growth appeared most rapid from birth to about 14 days of age, slowing at about the time of weaning. Growth was nearly linear from weaning at 21 days through ca. 50 days of age, with daily increases of 1.90 and 1.33 g/day for males and females, respectively. Growth continued at a slowly decreasing rate through ca. 90 days of age, and tapered considerably thereafter so that further increases in mass were minimal after 200 days of age (Fig. 1). Measurements of mass on each sex fit Gompertz growth curves well, especially over the range of ages (21–90 days) for which the effects of inbreeding on growth were assessed. Across all 851 weights of males, 95.9% of the variation in log (mass) was explained by the equation:  $\log(\text{male mass}) = 5.6281 - 3.9045 \times \exp(-0.02285 \times \text{age})$ . Across all 936 weights of females, 92.9% of the variation in log (mass) was explained by the equation:  $\log(\text{mass of female}) = 4.9741 - 3.0778 \times \exp(-0.02621 \times \text{age})$ . Because growth was especially rapid during the first 2 weeks, but the Gompertz curves were de-

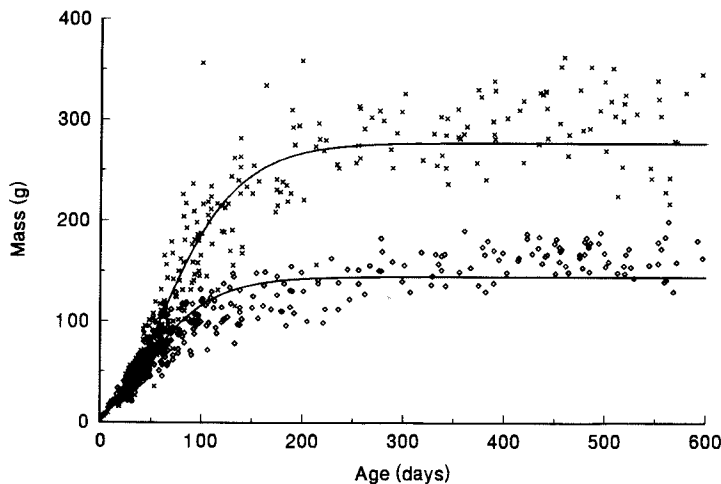


FIG. 1.—Mean mass of males (x's, upper line) and of females (diamonds, lower line) in litters of *Rattus villosissimus*. Data for weights beyond 600 days of age are not shown. Lines are sex-specific Gompertz growth curves, fitted by minimizing least-squares deviations from the non-linear model, with data weighted by the number of animals contributing to each litter same-sex mean.

terminated largely from post-weaning measurements, the Gompertz equations slightly over-predicted the birth weights (predicted mass: 5.6 and 6.7 g for males and females, respectively; measured mass: 4.5 and 4.4 g for 17 males and 14 females, respectively).

During the period of rapid post-weaning growth (21–90 days of age), linear and log-linear models fitted the data as well as did the Gompertz curves—with 82.7 and 78.5% of the variance in male and female mass, respectively, explained by simple linear growth, 79.9 and 75.0% explained by the log-linear model, and 81.7 and 77.6% explained by the Gompertz models. Over the entire dataset (animals measured from 0 to almost 1,200 days of age), linear and log-linear models fitted the data less well (61.7 and 68.5% of the variance in mass of males and females, respectively, explained by linear models, and 43.1 and 47.2% explained by log-linear models versus 95.9 and 92.9% for the Gompertz models).

The range of weights reported by Carstairs (1980) for *R. villosissimus* trapped in the wild (57–340 g for males; 39–240 g for females) is comparable to that seen in this study, and the sizes of 12 captive-born animals measured at 3, 21, and 90 days by

Carstairs (1980) also fell within the range of values in this study and close to the Gompertz curves. The parameters of the Gompertz curves for male and female *R. villosissimus* fell near the middle of the range of values reported by Zullinger et al. (1984) for seven other species of *Rattus*.

After removing the effect of age, by assessing deviations from Gompertz curves, neither the mean mass of females in a litter nor the mean mass of males in a litter was found to be significantly affected by inbreeding (Table 1). There was a significant negative effect of inbreeding on the total mass of litters (extrapolated to a 44% reduction predicted in fully inbred litters at 41 days of age, the mean age at which mass was measured), but much of this effect could be accounted for by the smaller initial number of offspring per litter (extrapolated reduction of 34%). After factoring out the effect of initial size of litter, the residual effect of inbreeding on age-adjusted mass of litter was not significant ( $P = 0.75$ ).

Although males averaged 19% larger than females by 41 days of age and 56% larger by 90 days of age, there was no association between the sex ratio of a litter and the age-adjusted mass of litters ( $r =$

TABLE 1.—Tests of effects of inbreeding on measures of reproductive performance in *Rattus villosissimus*.

Variable	n <sup>a</sup>	Predicted values		P
		at F = 0	at F = 1	
Number born <sup>c</sup>	294	6.46	4.26	0.001
Number weaned <sup>c</sup>	291	5.30	3.78	0.025
Viability <sup>d</sup>	291	0.920	0.973	0.228
Mass of females <sup>e</sup>	158	53.6	48.7	0.220
Mass of males <sup>e</sup>	158	62.1	58.0	0.428
Mass of litter <sup>e</sup>	170	312.2	174.7	0.015
Sex ratio				
(% female)	291	51.9	50.0	0.808

<sup>a</sup> Sample sizes (n) are numbers of litters measured. Three litters sacrificed at an early age were not included in analyses of number weaned, viability, or sex ratio. Not all litters were weighed.

<sup>b</sup> Predicted values at F = 0 and F = 1 and probability values are from regressions of the effect of inbreeding on the variables.

<sup>c</sup> Square-root transformed prior to analysis, with predicted values back-transformed from model estimates.

<sup>d</sup> Arcsin square-root transformed prior to analysis, with predicted values back-transformed from model estimates.

<sup>e</sup> Regression analysis was performed on the deviations of log (mass) from the Gompertz curves, with the predicted values back-transformed and given in grams for animals at 41 days of age.

0.04,  $P = 0.642$ ). This occurred because there was a relationship between the percentage of females and the number of young in a litter, both at birth ( $r = 0.16$ ,  $P$

$= 0.008$ ) and at weaning ( $r = 0.14$ ,  $P = 0.021$ ). After removing the effect of inbreeding, litters consisting of all females averaged 1.4 more young at birth and 1.2 more young at weaning than did litters of all males (estimated from regressions of square-root-transformed sizes of litters against F and sex ratio). As shown in Fig. 2, there was a switch from male-biased litters ( $44.7\% \pm 3.2 SE$ , females) to female-biased litters ( $53.8\% \pm 1.6 SE$ , females) when size of litter exceeded four. Sex ratio had no significant effect on the viability of litters, the age-adjusted mass of female offspring, age-adjusted mass of male offspring, or the age-adjusted total mass of litters.

The relationship between sex ratio and number of young born occurs in both inbred and non-inbred litters, but the size of litter at which the mean sex ratio switches from being male-biased to female-biased is decreased in more inbred litters. This resulted in a small, non-significant effect of inbreeding on sex ratio after the effect of the size of litter was removed. Fully inbred litters were extrapolated to have 1.9% fewer females when the size of the litter was not considered (Table 1), but 2.1% more fe-

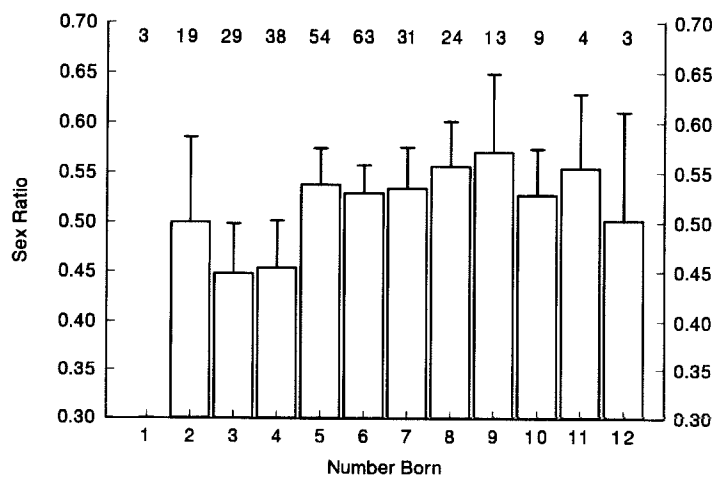


FIG. 2.—Mean sex ratio (proportion of weaned animals that were female), with standard error bars, of litters of various sizes for *Rattus villosissimus*. Not shown on the figure, three singleton litters consisted of males (SR = 0.0). Number of litters of each size is given above each bar.

males when compared to non-inbred litters with the same number of young.

#### DISCUSSION

**Reproductive rate.**—*Rattus villosissimus*, the long-haired rat or mayaroo, is essentially a grassland species, and its range includes much of inland Queensland and the Northern Territory, extending into north-eastern Western Australia, northwestern New South Wales, and northeastern South Australia (Watts and Aslin, 1981). Seemingly rare during times of drought, these animals multiply rapidly under conditions favoring growth of vegetation. From time to time their populations reach so-called plague proportions, with vast numbers of rats moving across the countryside as food, water, and suitable burrowing sites diminish. Starvation, disorientation, predation, cannibalism, crowding, and stress have all been invoked as contributing to the ultimate, and often sudden, population declines (Watts and Aslin, 1981). Although details of the social system and population structure in the wild are not known, it is possible that a cycle of irruptions interspersed with years of rarity would lead to frequent local inbreeding with occasional outcrossing among populations during post-irruption migrations. The progenitors of animals used in this study were collected during a local irruption.

The mayaroo breeds well in captivity, and the following descriptions are based on animals raised at Smith College. The weights of 90 specimens within the first 24 h (day 0) average 4.2 g. By day 4, the pinnae are free. The digits, fused at birth, are free by day 9. By day 11, the lower incisors are through the gums, and the upper incisors are erupting. The eyes and ears are completely open by day 18. By day 19, the young are fully furred below as well as above. Gestation period, as reported earlier for this colony (Taylor and Horner, 1973a), ranges from 22 to 23 days. Post-partum mating occurs commonly, and lactation may delay a subsequent parturition by  $\geq 9$

days. Size of litter, based on 295 litters, averaged 5.62, ranging from one to 13, with a sex ratio at weaning of 53% females ( $n = 795$ ) to 47% males ( $n = 708$ ). The earliest age of breeding was 64 days, while the earliest parturition occurred at 57 days, indicating impregnation at 34 or 35 days of age. One female produced 14 litters in  $<20$  months. Of her 97 offspring, 84 survived until weaning at 21 days of age. The only exception observed to the usual mammary formula of  $3 + 3 = 12$  (the maximum formula among Australian species of *Rattus*) is that of a single female exhibiting  $4 + 3 = 14$ .

Watts (1979) suggested that Australian *Rattus* have largely pushed adaptations for rapid reproduction (short estrous cycles, short gestation, and rapid sexual maturity) to the physiological limits, with perhaps the only scope for further increases in reproductive rate being in yet larger litters. *R. villosissimus* has among the largest litters (Taylor and Horner, 1973a) and is perhaps capable of the fastest rates of increase of the Australian rats. *R. villosissimus* from a rich agricultural region in Western Australia have been reported to have a mean of 12.6 offspring/litter (King, 1994), suggesting that over ecological or evolutionary time the species may be able to exploit good conditions by increasing size of litters. Of the several measures of reproductive performance available from the breeding records of the lab colony at Smith College, we found inbreeding depression only in size of litter and in direct correlates of the initial size of litter. Thus, there remains substantial genetic variance for size of litter in the species, with dominance or over-dominance for larger sizes of litters.

Interestingly, a reduction in size of litter that occurs with inbreeding is not necessarily maladaptive. In the highly variable natural environment of *R. villosissimus*, cumulative levels of inbreeding could presumably reach maxima during the later generations of population irruptions, when population densities are approaching their

peaks, but before dispersal of the local population. At such a time, increased competition may favor females that reduce the numbers of offspring per litter while maintaining the size of individual offspring, rather than continuing to produce large numbers of offspring that might have decreased growth or survival. Outcrossing would occur during dispersal and colonization of new habitats, and inbreeding levels would be low during early generations of population growth. Under such conditions, maximum production of offspring might be favored.

Studies of laboratory rodents and domesticated livestock have demonstrated that inbreeding often causes a depression in growth rates and body sizes of adults (Falconer, 1989; Wright, 1977). Laird and Howard (1967) and Kidwell et al. (1969), for example, reported that most of the parameters of the Gompertz growth curve differed between inbred strains of mice and their  $F_1$  hybrids. Hybrid mice reached maximum growth more quickly and attained a greater maximum growth, but also had a faster exponential decay in the growth rate so that the asymptotic body size was not consistently greater than in the inbred parental strains. White (1972) reported significant depression of growth to weaning at 21 days in laboratory mice that were progressively inbred, and his more inbred mice did not subsequently recover their decrements in weight. In our study, there was no difference in the shape of the Gompertz growth curve at several levels of inbreeding, and rats weighed at various times during the period of rapid growth showed no change in age-adjusted size as they were increasingly inbred.

Studies of the inbreeding in non-domesticated species of mammals have most often focused on the impact on survival of juveniles (e.g., Lacy et al., 1993; Ralls et al., 1988). Yet the impacts of inbreeding on mating ability, fecundity, and other components of fitness might be greater than the impact on survival rates (Brewer et al.,

1990; Lacy et al., 1996; Miller and Hedrick, 1993; Wright, 1977). In this study, inbreeding had no impact on survival of juveniles. Sizes of litters were substantially reduced at birth, and this effect of inbreeding carried through to result in fewer offspring weaned per litter.

There are several genetic mechanisms that could cause inbreeding depression, and they have different long-term consequences (Charlesworth and Charlesworth, 1987; Hedrick, 1994). Over many generations of inbreeding, deleterious recessive alleles could be purged from the population by natural selection, so that further inbreeding might have little or no negative effects, and the population would recover fitness. If over-dominance for fitness is widespread, however, deleterious alleles cannot be purged by selection (because all alternative alleles are deleterious when homozygous), and, unless strong balancing selection prevents losses of heterozygosity, the population would progressively lose fitness as it became inbred. There is relatively little evidence for purging of the genetic load (alleles causing fitness depression when homozygous) among studies of the effects of inbreeding in mammals. For example, White (1972) reported nearly linear effects of inbreeding on growth rate of laboratory mice, and the non-linear effects he observed showed an increased severity of inbreeding depression at higher levels of inbreeding attained by multiple generations of full-sibling mating. Similarly, Brewer et al. (1990) reported no diminution of the effects of inbreeding following multiple generations of inbreeding in *Peromyscus*. The zoo populations examined by Lacy et al. (1993) also showed no decrease in the slope of viability versus inbreeding at higher levels of inbreeding. In our laboratory colony of *R. villosissimus*, we detected no significant non-linearity in the effect of inbreeding, even as the population progressed to inbreeding levels as high as  $F = 0.80$  over 25 generations. If the reduction in size of litter with inbreeding were due to a few genes of



major effect (e.g., lethal alleles affecting embryos), then a substantial portion of the genetic load should have been purged (Hedrick, 1994). Thus, it would seem that the genetic load affecting size of litter in this species consists either of overdominant loci, which cannot be purged of the load, or of many alleles of small effect, which would be ineffectively purged in a small population subjected also to substantial genetic drift.

*Sex ratio.*—We found a complex relationship between the number of young born in a litter, the sex ratio of the litter, and inbreeding. Fisher (1930) suggested that natural selection should favor an equal total investment by a population in male and female offspring, because females (collectively) and males (collectively) must have equal reproductive success. If one sex is larger and, therefore, likely to be more costly to rear, then it would be adaptive to produce fewer of the more costly sex. In *R. villosissimus*, males are much larger than females (Fig. 1), but a significant sex-difference in body mass appears only subsequent to weaning (at ca. 21 days of age). There was a slight bias in favor of females in the overall sex ratio, but it did not differ significantly from equal (Table 1). However, there was a significant relationship between the sex ratio of a litter and the number of young born (Fig. 2). Although the shift in sex ratio was modest, litters with fewer offspring tended to contain a higher proportion of males (45% females in smaller litters, 54% females in larger litters). Given the faster growth rate of males, but the smaller number of offspring weaned in litters that are predominantly male, the mean mass of male-biased litters would be expected to catch up to the mean mass of female-biased litters at ca. 45 days.

Because sexes of offspring were recorded at the time of weaning, a shift in sex ratio with larger litters could result from greater mortality of females in small litters and greater mortality of males in large litters, or from an initial bias in favor of females fol-

lowed by higher mortality of females (producing some small litters with a deficiency of females). However, the relationship between the number of young born and the sex ratio of the litter was unaffected when only those litters that suffered no deaths of infants were considered. Thus, the changing sex ratio at different sizes of litters was due to a bias at conception or differential mortality during gestation. Other researchers have reported similar shifts toward more male offspring in smaller litters in a number of mammalian species (Clutton-Brock and Albon, 1982). Whether such shifts are adaptive, perhaps allowing relatively constant investment per litter even as the sex ratio varies, or are a non-adaptive consequence of differential early mortality cannot be determined.

There has been debate as to whether any species of mammal has the ability to adjust the sex ratio of progeny as an adaptive response to changing environmental conditions. Sex ratios deviating from equality are uncommon in mammals (Clutton-Brock and Albon, 1982), although one of the few species reported to have a significantly male-biased sex ratio at birth is *Rattus exulans* (Harrison, 1955), a species related to *R. villosissimus* and found on New Guinea and on a few small islands along Australia's northern coast (Taylor and Horner, 1973b). Striking regional and temporal variations in the sex ratio of wild *R. villosissimus* have been reported (Carstairs, 1976; Predavec and Dickman, 1994), but these may be due to sex differences in post-weaning mortality or likelihood of capture.

Trivers and Willard (1973) and Myers (1978) proposed, for different reasons, that mammals in which there is a sexual dimorphism in size might adaptively shift the sex ratio of progeny in response to maternal condition. *R. villosissimus* is presumably polygynous, and adult males are typically 50–100% larger than females. Inbreeding caused a substantial decrease in fecundity in *Rattus* in this study; thus, inbreeding might be a stress that could lead to an adap-

tive shift in sex ratio. Inbred females might not be as able to produce males that can successfully compete for mates, or inbred males might be less able to acquire mates. Therefore, when inbreeding, it might be adaptive for females to shift toward production of females in preference to males (Trivers and Willard, 1973). Moreover, if males, being larger, are more costly to rear, then females stressed by inbreeding might shift the sex ratio of offspring toward the less costly females to maintain numbers of offspring reared (Myers, 1978). Finally, a shift in sex ratio might be a non-adaptive consequence of greater mortality of the more costly sex (Clutton-Brock and Albon, 1982).

Contrary to the concordant predictions of the above theories, inbreeding did not lead to a significant shift in the sex ratio in our study. With progressive inbreeding, litters became smaller, but the overall sex ratio remained indistinguishable from 50% males. Because of the relationship observed between the sex ratio and size of the litter, this constancy of sex ratio under inbreeding had to be achieved by a downward adjustment in the size of the litter at which the sex ratio shifted from a male bias to a female bias. This could occur simply by a sex-unbiased reduction in size of litter with inbreeding. Inbreeding did not cause a decrease in body size nor an increase in pre-weaning mortality in the colony of *R. villosissimus*. Therefore, inbred progeny, while fewer in number, might be as successful in acquiring mates as are non-inbred rats. Myers' (1978) hypothesis that females under stress would shift the sex ratio of offspring toward the smaller females to maintain numbers of offspring (while sacrificing size) was not supported by this study. Inbred *R. villosissimus* produced fewer offspring, but this presumably deleterious effect of inbreeding might allow females to rear inbred progeny that have as rapid growth, as good survival, and the same sex ratio as do non-inbred young.

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