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22. We thank D. Kerr for suggesting this study and O. Pellett and F. L. A. Becker for technical assistance. Supported by PHS grant GM 17702 and American Heart Association grant 71-981. J.A.S. was an Established Investigator of the American Heart Association. W.A.K. is on leave of absence from the Department of Pediatrics, University of Heidelberg, Federal Republic of Germany, supported by the Deutsche Forschungsgemeinschaft (Kr328/5).

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Peromyscus: Effect of Early Pairing on Reproduction

Abstract. *Sibling mating in prairie deer mice* (Peromyscus maniculatus bairdi) *results in poor reproductive performance. Siblings experimentally paired before puberty exhibit delayed reproduction when adult. A behavioral mechanism is involved in this reproductive delay, since prepubertal familiarity also delays reproduction in nonsibling pairs. Such a reproductive delay may act to reduce inbreeding depression and regulate population growth.*

In natural populations of rodents the panmictic breeding unit is small, and there is genetic isolation between local populations, or demes (*1*). In *Peromyscus maniculatus*, a North American species, deme size is further limited because juveniles travel in sibling groups and disperse only short distances from their parents' home range (*2*). Such conditions may result in a high incidence of inbreeding. Since close inbreeding can lead to inbreeding depression, a serious reduction of various components of fitness (*3*), many species possess some mechanism to reduce the probability of inbreeding. Pregnancy block (*4*) may serve this function in mice but at the cost of energy and time expended in mating and the initial stages of pregnancy. A genetic mechanism in which males mature later than females would reduce inbreeding without this waste of time, energy, and gametes (*5*). However, in *P. maniculatus* such a mechanism would have minimal effect since the difference in age of maturation is at most a few days. A behavioral phenomenon which reduces inbreeding by inhibiting consanguineous mating is the incest taboo in man (*6*). To the best of my knowledge, a functional incest taboo has not been reported in any rodent species.

In the study reported here sibling pairs of *Peromyscus maniculatus bairdi* exhibited delayed breeding (*7*). This delay in breeding apparently results because a nonsexual relationship formed before puberty interferes with the later establishment of a sexual relationship. Although the exact mechanism of this interference in *Peromyscus* is as yet unknown, there is evidence that in other genera behavioral factors are involved. For example, in male rats play behavior habits established before puberty interfere with adult copulatory behavior (*8*), and in humans childhood association interferes with the later establishment of a sexual relationship (*9*).

Four experimental groups of bisexual pairs of mice were used (*10*). The first two groups were paired at 21 days of age. Of these, one group consisted of sibling pairs ("early siblings," $N = 30$ pairs) and one group of nonsibling pairs ("early nonsiblings," $N = 30$ pairs). The other two groups were paired at 50 days of age and also consisted of sibling pairs ("late siblings," $N = 31$ pairs) and nonsibling pairs ("late nonsiblings," $N = 29$ pairs). All individuals mated late were maintained in unisexual sibling pairs until mated. The two ages for mating made it possible to compare pairs mated before sexual maturity with pairs mated after sexual maturity. In natural popualtions of *P. m. bairdi* sexual maturity in females has been reported as early as 35 days of age (*11*). In the study reported here, sexual maturity may have been attained as early as 37 days of age: this subspecies has a gestation period of 21 to 23 days (*5, 12*), and the youngest early-mated pair to produce a litter was 60 days old.

Whenever possible, experimental animals were chosen from litters that contained at least two males and two females. From each such litter one sibling pair was mated and the remaining male and female were mated with individuals from a similar litter. Thus, a litter was represented in both the sibling and nonsibling matings to reduce the effect of any difference in fertility between litters. Members of nonsibling pairs were from litters born not more than 2 days apart. All animals were from the second laboratory-reared generation descended from wild-caught stocks.

All mice were housed in clear plastic cages (15 by 30 by 15 cm) at 20° ± 1°C and 20 to 70 percent relative humidity; they were on a daily cycle of 15 hours light and 9 hours dark. Under these conditions, *P. m. bairdi* breeds throughout the year with a peak from summer through early fall (*13*). My experiment was begun in early fall and continued until fall of the following year.

After mating, females were examined for any indication of pregnancy at least once a week. Pregnant females were examined twice a day until the litter was born. The litter was not disturbed until 24 hours after its birth, when the number, sex, and average weight of

the surviving offspring were determined. Pups that died during the first 24 hours were counted as part of the litter but, because they were dehydrated or partially cannibalized, were not included in the calculations of average weight. The number and weight of the offspring that each pair reared to weaning age were also recorded. Although these data were collected for subsequent litters, only the data from the first litter produced by each pair are presented here. After 1 year the reproductive organs of nonreproductive individuals were weighed and compared with those of reproductive individuals.

The results of this experiment were analyzed for inbreeding depression by comparing the reproductive performance and offspring of the sibling pairs with those of the nonsibling pairs. The influence of prepubertal familiarity on reproductive performance was determined by comparing the performance of the early-mated groups with that of the late-mated groups. Finally, this experimental design allowed the two factors, time of mating and relationship of the pair, to be analyzed simultaneously to determine which one had the greatest influence on reproductive performance (*14*).

Examination of the offspring indicated that sibling mating in *P. m. bairdi* results in inbreeding depression. Analysis of variance shows that at 1 day of age the litters of sibling parents consisted of fewer individuals ($P < .05$), which were lighter in weight ($P < .005$) than those of nonsibling parents. Sibling pairs had a mean of 4.1 ± 0.2 (standard error) and nonsibling pairs a mean of 4.8 ± 0.2 young per litter. The mean weight of young per litter was 1.73 ± 0.04 g for the sibling pairs and 1.85 ± 0.03 g for the nonsibling pairs. The age of the parents at mating did not affect the number or weight of 1-day-old young. At weaning age (21 days) differences in mean litter size and weight were no longer significant, because among the litters of sibling parents infant mortality was greatest in the small, light litters and only the large, heavy litters survived to weaning. These surviving litters were similar in size and weight to those of nonsibling parents. Nonsurviving offspring did not exhibit any obvious physical defects at birth: it is not known if they died because of illness or because they were abandoned or killed by their parents.

The overall reproductive performance of sibling pairs was poorer than that of nonsiblings. The proportions of reproductive and nonreproductive pairs were not the same for all groups (Fig. 1), with the early siblings having significantly fewer reproductive pairs than the other three groups ($\chi^2 = 8.66$, d.f. $= 3$, $P < .05$). The proportion of reproductive pairs in the late sibling group was similar to that in both nonsibling groups and to the proportion of fertile females previously reported in this subspecies (*12*). However, the proportion of litters in which at least one pup was reared to weaning age was significantly lower in both sibling groups than in the nonsibling groups ($\chi^2 = 11.81$, d.f. $= 3$, $P < .01$): early siblings, 13/20; late siblings, 15/27; early nonsiblings, 25/28; and late nonsiblings, 22/25. Therefore, both groups in which siblings were mated were subject to inbreeding depression (*3*); although late siblings produced more litters than early siblings, they reared only about the same number of litters to weaning age. There were no obvious anatomical reasons for the apparent lack of fertility in any nonreproductive individuals (*15*).

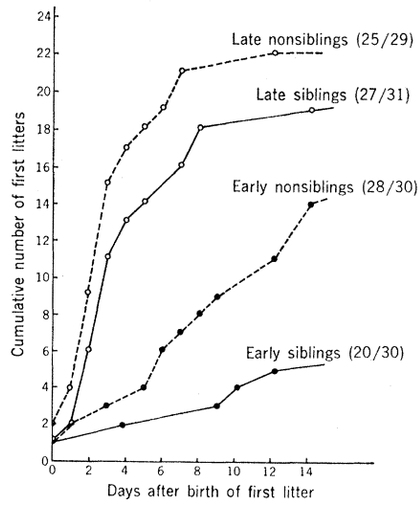

Fig. 1. Rate of production of first litters in the four treatment groups during the first 2 weeks after each group began reproducing. Fractions in parentheses indicate the proportion of pairs in each treatment group that produced litters within 1 year.

That early pairing results in delayed reproduction is shown in a comparison of rates of reproduction of the groups. Because early-mated pairs were exposed to each other longer than late-mated pairs, it was necessary to establish an equivalent point of origin to compare reproductive rates. The zero point was defined as the age of the mother on the day of birth of the first litter born in each group (*16*). The age of this youngest mother in each group was then subtracted from the age on the day of birth of their litters of the other mothers in that group. Thus, the birth dates of all the litters in a treatment group are expressed as the number of days following the birth of the first litter in that group (Fig. 1). Analysis of variance shows that the early-mated pairs of both siblings and nonsiblings reproduced at a significantly slower rate than the late-mated pairs ($P < .01$). Early-mated pairs had their litters 47.9 ± 8.7 days after the groups began to reproduce, while the late-mated pairs had their litters in only 19.3 ± 6.4 days (*17*). Differences in reproductive rate between siblings and nonsiblings and interaction effects between age of mating and genetic relationships were nonsignificant. This evidence supports the hypothesis that early pairing tends to interfere with reproduction. That this is a behavioral phenomenon is shown by the delay in reproduction in prepubertal matings regardless of the genetic relationship, sibling or nonsibling, of the individuals involved.

The fact that sibling mating does lead to inbreeding depression suggests a function for the apparent incest taboo revealed here. Since sibling mating results in a wastage of gametes, a behavioral mechanism that reduces this loss must be of selective advantage and would be maintained in the repertoire of a species. Such a mechanism, by at least delaying mating between individuals as familiar as siblings, would reduce inbreeding and allow an extended nonreproductive period during which nonfamiliar, nonsibling mates could be encountered.

In the event that no nonfamiliar mates appear, mating between siblings may eventually occur. However, even after such a sibling mating, if a nonfamiliar male appears, the first pregnancy might be blocked (*4*), allowing for a successful mating involving the nonfamiliar male. Clearly, prepubertal familiarity can regulate reproductive performance.

Delayed reproduction among familiar individuals also may serve as a population regulatory mechanism by increasing the time between successive generations. In natural populations of *P. m. bairdi* the young leave their natal home range in sibling groups (*2*), and generation time is increased because they may not mate until they meet unfamiliar individuals. The low level of repro-

duction among individuals born into confined laboratory populations (*18*) may result partly because these animals are familiar with each other before puberty.

The following conclusions can be drawn from the results of this experiment: (i) Sibling mating results in inbreeding depression in *P. m. bairdi*. (ii) Siblings paired at weaning age do not reproduce as readily when mature as do siblings paired when sexually mature. This may reduce the probability of inbreeding in mouse populations. (iii) The delay in reproduction due to prepubertal familiarity is independent of genetic effects since nonsibling pairs also exhibit it. The exact mechanism regulating this delay in reproduction due to familiarity of the pair is not yet known. Such a delay may result from any of several factors, such as interference of other behaviors with copulatory behavior, delayed sexual maturation, blockage of fertilization or implantation, resorption of embryos, or early abortion and ingestion of embryos. Elucidation of this mechanism, including the role played by pheromones (*19*) in delaying reproduction in familiar pairs, should be the object of further research.

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15. Weights of testes and seminal vesicles or ovaries and uteri did not differ between reproductive and nonreproductive individuals. Of the nonreproductive females, 13 had not recently ovulated, 6 had corpora lutea, and 1 was pregnant with two embryos.
16. The age in days of the youngest female to give birth in each group was: early siblings, 60; early nonsiblings, 61; late siblings, 73; and late nonsiblings, 74.
17. The difference in reproductive performance developed within the first 2 weeks. By day 9 (the median value for all groups combined) the early-mated pairs had produced only 25 percent (12/48) of their litters, while the late-mated pairs had produced 75 percent (39/52) of their litters.
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Antihistamines Block Radiation-Induced Taste Aversions

Abstract. *When rats are treated with an antihistamine prior to being given sublethal doses of ionizing radiation, the formation of a conditioned saccharin aversion is completely inhibited. A profound aversion could be conditioned with histamine diphosphate as the aversive stimulus. The increase in histamine production after radiation exposure represents the physiological basis of radiation-induced taste aversions.*

Garcia, Kimeldorf, and Koelling (*1*) found that a taste aversion to saccharin-flavored water could be conditioned in rats by pairing consumption of the sweetened fluid with a 37-r whole body exposure to gamma rays. Similar taste aversions have been conditioned by pairing saccharin intake with injections of lithium chloride (*2*), apomorphine (*3*), cyclophosphamide (*4*), and many other poisons. All of these treatments have been presumed to cause the animal to become "sick," and the pairing of sickness with intake of the novel saccharin solution resulted in the subsequent conditioned taste aversion. The injections of lithium chloride, apomorphine, or cyclophosphamide make the rats ill, and these rats can easily be discriminated from sham-injected controls. With whole body x-ray and gamma-ray exposures up to 100-r (*5*), the rats do not appear to be sick and cannot be distinguished from sham-exposed controls except by the subsequent aversion to the taste solution. This subtle and interesting reaction of the rat to the radiation stimulus was first demonstrated in 1955, and the physiological basis of a radiation-induced taste aversion has been the topic of considerable research.

The aversion phenomenon was formerly attributed to gastrointestinal disturbances (*6*), but considerable doubt has been cast on this explanation (*7*). Partial body exposures have shown that it is not necessary to irradiate the head of the rat in order to produce the taste aversion, thus eliminating vision, olfaction, taste, or audition as necessary for conditioning the aversion (*8*).

The studies by Hunt and his coworkers (*9*) with parabiont pairs of male rats indicated that some humoral factor may be involved in conditioning the taste aversion. When the shielded (nonirradiated) partner of the parabiotic pair was allowed to drink saccharin 30 minutes after the nonshielded partner received a whole body exposure of 360-r, the shielded partner avoided the saccharin solution during a saccharin-water preference test administered 24 hours later.

We have shown that the maximal saccharin aversion develops 30 to 90 minutes after the onset of the radiation exposure (*10*), implying that the aversive consequence of the irradiation reaches a peak during this interval.. The histamine concentration in the blood of rats exposed to 600 r of x-rays reaches a peak 60 to 120 minutes after the exposure (*11*). The purpose of the experiments reported below was to determine if the injection of an antihistamine prior to radiation exposure would inhibit the formation of a saccharin aversion, thus providing evidence for a causal relation between aversiveness of the irradiation and radiation-induced histamine production.

Chlorpheniramine maleate was chosen as the antihistamine because it is an active histamine antagonist, but it has a minimum of undesirable side effects (*12*). Naive male albino rats ($N = 28$) were placed on a 23.5-hour water de-