around the organizer. After 4 to 7 hours of incubation in ribonuclease, several puffs are induced. The efficiency of induction increases with the duration of the incubation time. After 7 hours the proportion of nuclei showing new puffs is 90 percent or more. At this time the chromosomes appear more dense, and there is some nonspecific stickiness. There are about 40 puffs induced, several puffs being present in each arm of every chromosome; the puffs occur always in the same chromosomal regions and are generally larger than puffs that occur normally during larval development.

The puffs induced in the left arm of the second chromosome are shown in Fig. 1. Puffs are induced in regions 22A, 29B, 30B, 31B, 32C-D, 38A, and 39C. These positions are deduced from the chromosome map of Krivshenko (*7*). The puffs at 30B, 31B, and 38A (previously called 2L14, -15, and -20) can also be induced by anaerobiosis, temperature shock, and uncouplers of oxidative phosphorylation (*4, 8*), and are in fact the only puffs induced in the genome by these agents. Not all the puffs appear simultaneously; there is a sequence of induction. On the left arm of the second chromosome the puffs appear in this order: 30B, 31B, and 38A; 32C-D; 29B and 39C; 22A.

F. M. RITOSSA
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*, and *Laboratorio Internazionale di Genetica e Biofisica,† Naples, Italy*
R. C. VON BORSTEL
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*

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Genotype and Sex Drive in Intact and in Castrated Male Mice

Abstract. *Male mice of two inbred strains and one hybrid strain were observed for sexual behavior for 42 consecutive days. Half the males of each strain were then castrated, and daily testing was continued until the ejaculatory reflex was lost. Strain differences were found in ejaculatory frequency both before and after castration.*

Male mice of different inbred strains differ significantly in several aspects of sexual behavior (*1, 2*). The time required to recover sex drive after an ejaculation is one of the variables which has been shown to be affected by genotype (*2*). More specifically, males of the inbred strain DBA/2J recovered sex drive (achieved a second ejaculation) in 1 hour while C57BL/6J males required a median recovery time of 4 days. Hybrid males resulting from a cross between the two inbred strains resembled DBA/2J males in that the time required to recover sex drive after an ejaculation was comparable. The previous studies, however, did not show that "fast-recovery" males were in fact capable of more ejaculations over an extended period of testing than were "slow-recovery" males.

One purpose of the present study was to test the foregoing hypothesis; the second purpose was exploratory in nature. One of the accepted generalizations from studies on sexual behavior is that the behavior of animals high on the phylogenetic scale is less dependent on gonadal hormones than is the sexual behavior of animals with a lower phylogenetic status (*3*). For example, the sexual behavior of experienced, male cats and dogs (*4, 5*) persists much longer after castration than does the behavior of experienced, castrated rats and guinea pigs (*6*). The second part of our experiment was designed to determine whether genetic differences within a species affect the persistence of sexual behavior after castration.

A total of 72 male mice was used, including 24 C57BL/6J males, 24 DBA/2J males, and 24 B6D2F$_1$ males. The last named strain results from crossing C57BL/6J females with DBA/2J males. Each male was housed with five other males of the same genotype in the intervals between the daily testing sessions.

Two hundred and fifty-two BALB/cJ females were used in the mating tests. Thirty-six of these females were brought into behavioral estrus each day by injections of estrogen and progesterone (*7*).

All animals were 9 weeks old at the beginning of the experiment. The animals were maintained on a reversed light-dark cycle with the light phase lasting 13 hours. The dark phase began 2 hours before the onset of testing which occurred under normal room illumination between 8:30 a.m. and 2:00 p.m.

Males were placed individually in plastic cylinders 25 cm in diameter and 50 cm in height. In the early stages of the experiment, males were allowed 30 minutes to adapt to the cylinder prior to the introduction of an estrous female. This 30-minute adaptation period became unnecessary as the males gained experience in the test situation. A given male was allowed from 5 to 10 minutes to initiate mating with the estrous female. If the male did not gain intromission during this interval, the female was removed

Table 1. Sexual performance of 24 intact males of each strain during 42 consecutive days of testing.

No. of ejaculators*	No. of ejaculations per ejaculator		Day of first ejaculation		Days between ejaculations	
	Median	Range	Median	Range	Median	Range
			C57BL/6J			
10	2	1–9	17	4–31	6	1–38
			DBA/2J			
22	15	4–28	3	1–36	2	1–19
			B6D2F$_1$			
24	15	5–27	2	1–32	2	1–9

* Males that achieved ejaculation.

and a second female was presented to the male. When a male "refused" all three females, he was scored as "negative" for that day. In order to achieve a "positive" score, the male was required to mate with one of the three females until ejaculation occurred. Occasionally, a male would cease copulating before ejaculation. Such a test was scored as "negative" if 40 minutes elapsed without an intromission.

Daily testing continued for 42 days at which point half the males of each strain were castrated. Castrate groups and noncastrate groups were matched within strains on the basis of number of ejaculations for individual animals. One C57BL/6J male and one DBA/2J male, both of which had previously copulated, died as a result of the operation. Daily testing was resumed 72 hours after castration. Daily tests then continued for each group until at least 14 days had elapsed without the occurrence of the ejaculatory reflex in a castrated male.

The results (Tables 1 and 2) may be briefly summarized as follows: (i) DBA/2J males exhibited higher sex drive than C57BL/6J males. This is illustrated in the four measures presented in Table 1. All four of the measures revealed statistically significant differences between the two inbred strains (*8*). (ii) The hybrid males, B6D2F$_1$, resembled the DBA/2J males in sex drive as defined by the measures presented in Table 1. They differed significantly from the C57BL/6J males, but not from the DBA/2J males, on all four measures. This finding agrees with a previous report concerning the "dominance" of the DBA/2J genotype over the C57BL/6J genotype in the determination of sex drive (*2*). (iii) The data of Table 2 illustrate "hybrid vigor" in the persistence of sex drive after castration. The hybrid males retained the ejaculatory reflex in greater numbers and for a longer time than either inbred parent strain.

This study has shown that genotype has a definite effect on the sex drive of the intact male mouse and that high sex drive seems to be a "dominant" character. The hypothesis, based on a previous study (*2*), that "fast-recovery" males are capable of more ejaculations over an extended period of testing than are "slow-recovery" males was supported.

Further, the study has demonstrated that the persistence of sex drive after castration varies with genotype *within* a species. Genetic homozygosity was associated with a rapid loss of the ejaculatory reflex in the castrated males; heterozygosity, on the other hand, resulted in a retention of this reflex for a maximum of 60 days after castration. This finding raises a question concerning the accepted generalization of an inverse correlation between dependence on gonadal hormones and phylogenetic status. For example, it may be hypothesized that the differences in decline of sexual behavior between carnivores and rodents is due not to their phylogenetic status, but rather to the amount of heterozygosity in the samples. The carnivores studied have been mongrel cats and dogs, while the rodent groups have been selected from relatively inbred laboratory colonies. Heterozygosity is doubtless greater in the carnivore groups and this may have accounted for the slower decline in sexual behavior. Support for this hypothesis is found in a recent study by Bruell (*9*) who tested several inbred strains of mice and their hybrids in a running wheel. Hybrid vigor occurred for all crosses, and the degree of hybrid vigor was found to be proportional to the amount of suspected heterozygosity.

Genetic differences may also account for the observation (*4, 11*) that mongrel cats fall into three different types on the basis of decline of sexual behavior after castration.

There is one previous study regarding the decline of sexual behavior in mice after castration which does not support the foregoing hypothesis. Champlin, Blight, and McGill (*10*) castrated hybrid males of the CD2F$_1$ strain (BALB/c females × DBA/2J males) and found that the ejaculatory reflex was lost within 1 week. The discrepancy between that study and ours may be due to (i) genetic differences (the CD2F$_1$ strain may remain homozygous at critical loci), (ii) maternal effects, or (iii) any of several procedural differences between the two studies.

The hypothesis that heterozygosity determines persistence of sexual behavior is testable. Should experiments support this hypothesis, the search for the underlying physiological differences will be greatly simplified as only one species need be used (*12*).

Table 2. Sexual performance after castration.

Castrates (No.)	Preoperative ejaculators (No.)	Postoperative ejaculators (No.)	Total ejaculations after castration (No.)	Day after castration on which last ejaculation occurred	
				Median	Range
		C57BL/6J			
11	4	0			
		DBA/2J			
11	10	3	3	3	3–8
		B6D2F$_1$			
12	12	9	42	28	3–60

THOMAS E. MCGILL
G. RICHARD TUCKER
Department of Psychology, Williams College, Williamstown, Massachusetts

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