pistillate parent with *H. gracilis* (Ariz.) as the staminate parent yielded 57 plants with $2n = 4$ and the *gracilis* karyotype and 48 plants with $2n = 5$ and the hybrid karyotype. A chi-square test for a 1 : 1 ratio of two- and three-chromosome eggs from the hybrid gives a probability $> .30$. A cross of *H. gracilis* (N.M.) as the pistillate parent and the hybrid as the staminate parent produced 106 plants with $2n = 4$ and the *gracilis* karyotype and 66 plants with $2n = 5$ and the hybrid karyotype. Both crosses thus demonstrated preferential segregation from the trivalent, with A_g going to one pole and C_t and D_t to the other.

Cytological studies of microsporocytes during meiosis in the hybrid showed no mechanism for the selection of two-chromosome gametes and pollen stainability was high (89 percent). Therefore, the deviation in the expected 1 : 1 ratio of $2n = 4$ and $2n = 5$ plants ($p < .01$) with the New Mexican *gracilis* race as the pistillate parent must be due to competition between two- and three-chromosome pollen grains. Whether the competition process acts at the time of pollen germination or during growth through the style is unknown.

Preferential segregation from a trivalent with and without chiasmata formation is known for the sex chromosomes of both animal and plant species (*4*). Preferential disjunction from a trivalent composed of two acrocentrics and a large metacentric genetically equivalent to, and derived, by previous centric fusion of the two acrocentrics was predicted more than 20 years ago for *Drosophila* (*5*). This was later demonstrated in hybrids between *D. americana* subspecies *americana* and subspecies *texana* which showed regular disjunction of two acrocentrics from a genetically equivalent metacentric without loss of fertility (*6*).

Because *H. gracilis* has only two pairs of chromosomes and because one of these undergoes preferential disjunction in the *gracilis* × tribivalens hybrid, a technique exists for determining which of the two linkage groups of *gracilis* is carrying a recessive marker gene. This technique is effective only if there is less than 50 percent recombination between the marker and the centromere of either C_t or D_t. Crossovers between B_g and B_t can be ignored if these chromosomes undergo random disjunction.

A theoretical cross of *H. gracilis* × tribivalens with recessive markers on the chromosomes of *gracilis* is shown in Fig. 2. Expected results of testcrossing the F_1 as the pistillate parent to *gracilis* homozygous for the markers may be summarized as follows. If the marker is introduced on A_g and no recombination occurs between the marker and the centromere, two equal classes of test cross progeny are expected: four-chromosome plants homozygous for the marker and five-chromosome wild types. Crossover classes would yield four-chromosome wild types and five-chromosome plants homozygous for the marker. If the marker is introduced on B_g, equal numbers of the following classes, including crossovers, should occur: four-chromosome plants homozygous for the marker; four-chromosome wild types; five-chromosome plants homozygous for the marker; five chromosome wild types.

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References and Notes

1. R. C. Jackson, *Science* **128**, 115 (1957).
2. ———, *Am. J. Botany* **46**, 550 (1959).
3. ———, *ibid.* **49**, 119 (1962).
4. C. P. Swanson, *Cytology and Cytogenetics* (Prentice-Hall, Englewood Cliffs, N.J., 1957), pp. 313–348; B. W. Smith, *J. Heredity* **46**, 226 (1955).
5. A. H. Sturtevant and E. Novitski, *Genetics* **26**, 517 (1941).
6. W. S. Stone, *Univ. Texas Publ. 4920* (1949), p. 18.
7. This study was supported by grant G 20864 from the National Science Foundation and by the General Research Fund of the University of Kansas. I thank R. Roy Johnson for many field collections.

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Chromosome Puffs in Drosophila Induced by Ribonuclease

Abstract. *Ribonuclease induces many puffs in salivary gland chromosomes of* Drosophila busckii. *The left arm of chromosome 2 was analyzed in detail; in this arm seven puffs were induced in a definite sequence.*

Chromosome puffs in dipteran salivary glands are structural modifications believed to be associated with gene action (*1*). Since RNA is synthesized in chromosome puffs (*2*), a series of experiments has been undertaken to attempt to elucidate puff structure and function in the presence of ribonuclease. This enzyme is known to enter cells, including salivary gland cells of *Drosophila*, and produce a number of alterations (*3, 4*). We have observed that ribonuclease can induce a large number of puffs.

Salivary glands of *Drosophila busckii* larvae reared at 25°C were excised and incubated in Ephrussi-Beadle solution (*5*) containing 3 or 5 mg of ribonuclease per milliliter (*6*). For the control, glands were incubated in Ephrussi-Beadle solution alone.

After about 1 hour of incubation, the nucleolus disappears almost completely—only a small remnant is found

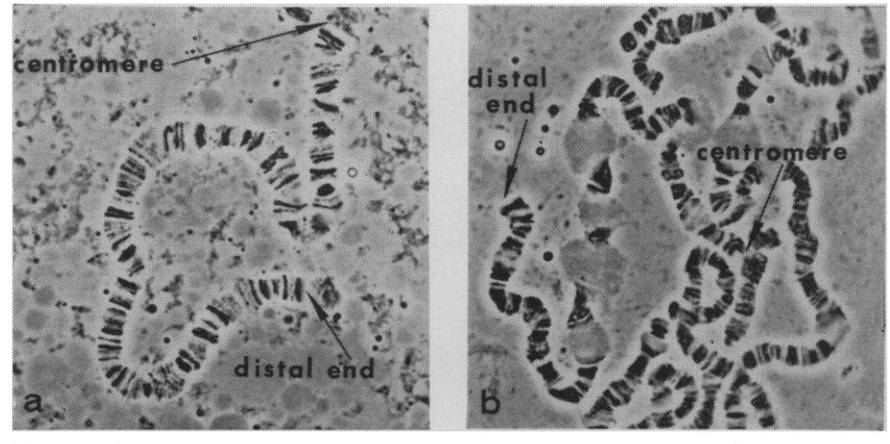

Fig. 1. Left arm of the second chromosome of *D. busckii*. *a*, A salivary gland smear in 45 percent acetic acid after 5 hours' incubation in Ephrussi and Beadle solution. *b*, A salivary gland smear after 5 hours' incubation in Ephrussi and Beadle solution containing 5 mg of ribonuclease per milliliter.

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.

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References and Notes

1. W. Beermann, *Am. Zoologist* **3**, 23 (1963).
2. C. Pelling, *Nature* **184**, 655 (1959); F. Ritossa, *Experientia* **18**, 571 (1962); G. T. Rudkin and P. S. Woods, *Proc. Natl. Acad. Sci. U.S.* **45**, 997 (1959); H. Swift, in *The Molecular Control of Cellular Activity*, J. M. Allen, Ed. (McGraw-Hill, New York, 1962), p. 73.
3. J. Brachet, *Biochemical Cytology* (Academic Press, New York, 1957); A. Jurand, I. Gibson, G. H. Beale, *Exptl. Cell Res.* **26**, 598 (1962); M. I. Perez del Cerro, *Exptl. Cell Res.* **24**, 580 (1961); F. Ritossa, *Atti Assoc. Gen. Ital.* **7**, 147 (1962).
4. B. P. Kaufmann and N. K. Das, *Chromosoma* **7**, 19 (1955).
5. B. Ephrussi and G. V. Beadle, *Am. Naturalist* **70**, 218 (1936).
6. Sigma, recrystallized five times.
7. J. D. Krivshenko, *Genetics* **48**, 1239 (1963).
8. F. Ritossa, *Exptl. Cell Res.*, in press.

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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.