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Fecundity of virgin versus non-virgin D. melanogaster males given varying numbers of females.

This experiment was designed to determine the number of females needed to exhaust a male's 24-hour supply of sperm, and to compare previously unmated with previously mated males. All flies were Canton-S raised at 25° C on cornmeal-molasses-yeast-agar food. At the time of the experimental matings (day 0) all flies were four days old. Virgin fathers were stored singly in vials from age 0 to age 4; non-virgin fathers were stored 10♂♂ to 300♀♀ in half-pint bottles from age 0 to age 4; virgin females were stored 200 per bottle from age 0 to age 3 at which time they were distributed into groups of 1, 3, 9, or 27♀♀ per vial. On day 0 one male was added to each of the female-containing vials (etherizing the male only), left for 24 hours, and then removed (without etherization). The females were not separated but were subcultured on days 4, 7, 10, 13, 16, 21, 26, and 33. A group of females mated to a single male, together with the subcultures of these females, will be designated a harem. The results are indicated below.

type of father	♀♀ in harem	replicates	fertile replicates	total offspring	offspring of all replicates per harem	offspring of all replicates per ♀	offspring of fertile replicates per harem	offspring of fertile replicates per ♀
virgin	1	12	6	1,605	134	134	268	268
virgin	3	12	10	5,185	432	144	519	173
virgin	9	12	12	9,073	756	84	756	84
virgin	27	11	11	11,788	1,072	40	1,072	40
		47	39	27,651				
non-virgin	1	12	2	513	43	43	256	256
non-virgin	3	12	8	2,232	186	62	279	93
non-virgin	9	12	12	4,532	378	42	378	42
non-virgin	27	12	12	5,549	462	17	462	17
		48	34	12,826				

It is seen that virgin males were more successful fathers than experienced males during the 24-hour test period: more harems were fertile and more offspring were produced on either a per harem basis or on a per fertile harem basis. Whether this advantage was gained by an increased likelihood of copulation, or by more sperm being transferred per copulation, or both, is unclear, although differences in the frequency of copulation, by itself, could account for the observations. This is supported by Mossige (Am. Nat. 1955) who found that 2-day-old virgin Canton-S males, when provided 10 females for 24 hours, could fertilize 8.5 ♀♀ on the average; under similar conditions, 2-day-old non-virgin males fertilized 4.6 ♀♀.

How many females are needed to exhaust a male's supply of sperm? The results of this experiment are equivocal. The total number of offspring increases throughout the range of females used, although it might seem that 9 females, and certainly 27, could exhaust a male's sperm even if any one female mated only once during the 24-hour period. The experiments of Lefevre and Parker (D.I.S. 1963), also based on total progeny counts, suggest that a male can be "saturated" by fewer than six females when the male is transferred daily over a five day period. Mossige (Am.Nat. 1955) showed that a virgin male of any age (up to 21 days), when provided with ten females, would fertilize about 8 of them during the first 24 hours after mating. With continued daily transferring to 10 new females, however, this number decreased to about 2 fertilized females per day. McSheehy (D.I.S. 1963) reported a male mating frequency of about 1.5 copulations per day when males (virgins?) were provided two females each for 70 hours, and 8.7 copulations per day when provided eight females each.

However, neither total progeny counts, nor number of females successfully inseminated, nor copulation frequency indicates directly the parameter of interest, namely, sperm depletion. If, say, five females can deplete a male's sperm supply, then providing more females may, nevertheless, increase the frequency of copulation (as McSheehy's experiment suggests), and therefore the number of fertilized females and the total progeny count. One need only suppose that, with an increased supply of females, each of the more frequently occurring copulations involves fewer sperm, but not fewer than the female can store and utilize. (A female generally stores far fewer sperm--less than 700--than the 3-4 thousand that a male can transfer

(Kaplan, et al, D.I.S. 1962; Kaufmann and Demerec, Am. Nat. 1942).) This view is supported by the data of Stromnaes and Kvelland (Hereditas, 1962) who showed that females inseminated by highly active males (those mating with all ten of the females provided by 12 hours) produce fewer progeny, on the average, than females inseminated by less active males. However, on a per male basis, the more active ones produced the greatest total number of progeny.

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Mutations induced at specific loci in motile sperm.

Adult virgin "Binsc" males 7-9 days old were X-rayed (3000r) and immediately mated to females bearing the "maple" chromosome (y ac sc pn w rb cm ct^6 ras^2 v g^2 f car) heterozygous with Ins(1)sc^{S1}, dl-49, y sc^{S1} v B. Mating was allowed for only 2-3 hours in order to sample fully mature (motile) sperm (Lefevre and Jonsson, Mut. Res. 1, 1964). F_1 B females were observed for mutations at the marked loci. Altogether, 3163 males were irradiated, 10,132 F_1 females were examined and 69 mutants were found.

The yellow mutants were considered apart from the rest because in the Binsc chromosome this locus is near a portion of heterochromatin and has been shown to be very frequently involved in rearrangements (especially small deletions) with one break in this heterochromatin. The mutation frequency of yellow in this condition is a good estimate of heterochromatic rearrangement frequency. The overall mutation frequency of the y locus was 8.67 x 10^{-7}/r, which is significantly higher than the mean overall mutation frequency found for the remaining loci (ac and sc excluded), which was 1.43 x 10^{-7}/1/r. (The overall frequency includes cases of mutant F_1 females which were inviable or sterile.)

In the table, the results are compared with those obtained by R. Valencia (unpublished) in a similar experiment where a mixture of spermatozoa was studied. In this case, sperm was collected during about 3 days after treatment of 3-5 day old males. Point mutations have been separated from chromosome rearrangements according to the viability and fertility of the mutants which were kept in stocks, the classifications being based upon past experience of Muller and the Valencias with these same loci. This classification will be checked by cytological analyses now in progress.

The results for the 10 "maple" loci observed (other than y) are in agreement with those of Lefevre (Genetics, 1967). That is, the increase in X-ray induced mutation frequency in motile sperm is due to an increase in chromosome rearrangements and not point mutations.

Mutations	Sperm	Av.mut.freq./locus/r x 10^7 (2)	Freq.(motile) / Freq.(mixture)
Point mut. 10 loci(1)	Motile	.4	.8
	Mixture	.5	
Chr. rearr. 10 loci(1)	Motile	.7	1.4
	Mixture	.5	
Chr. rearr. (yellows)	Motile	3.7	1.5
	Mixture	2.4	

(1) pn w rb cm ct ras v g f car

(2) Frequencies include only those mutations of which stocks could be made.

Chromosome rearrangements involving the y locus show a relative increase in frequency in motile sperm which is quite similar to that observed for the other loci. This suggests that the X-ray sensitivity (either increased breakage or impaired ability to repair) of heterochromatic regions and of euchromatic regions is equally enhanced in fully mature sperm. This situation is quite different from that found in female germ cells, in which the increase in yellows due to heterochromatic rearrangements in stage 14 as compared with stage 7 oocytes was about 88 times greater than the increase in other loci (Valencia and Valencia, Rad. Res. 14, 1961, and R. Valencia, Genetics 52, 1965).