All collected flies were alive and appeared vigorous at this time, 3 days after the ether or CO_2 anesthetization. Male and female pairs were aspirated from their holding vials into observation vials containing media seeded with liquid yeast two days previously. A block of pairings consisted of an ether-treated male with an ether-treated female, an ether male with a CO_2 female, a CO_2 male with an ether female, and a CO_2 male with a CO_2 female. Two blocks were started at each observation period by adding all males, then all females within 5 min of each other, or the reverse order. The time of initial pairing, time of male mounting female and time of dismounting were recorded for each pair to the nearest half-minute.

The factorial analysis of variance for latency to mounting and copula duration are presented in Table 1. These two measures were transformed to their common logarithms for analysis to reduce the correlation of group means with variances. Within group variances are homogeneous, as determined by an F_{max} test (F_{max} = 1.45, Df = 4,14 and F_{max} = 2.26, Df = 4,13 for mounting latency and copula duration, respectively). Female anesthetic treatment significantly affected latency to mounting, and had the largest, but nonsignificant effect on copula duration. Male treatment and the interaction of treatments are nonsignificant components of variance.

Table 2. Mean effects of female anesthetic treatment on mating components.

Treatment	Mounting latency	Copula duration
Ether	11.5 min	22.21 min
CO_2	18.2 min	20.93 min

The effect of carbon dioxide treatment on females is to increase latency to mounting and decrease copula duration relative to ether treatment, as indicated in Table 2. These effects suggest that carbon dioxide use in virgin collecting may have a long-term effect on reproductive responses of females.

Supported by NIH AG02035.

References: Ashburner and Thompson 1978, in: The Genetics and Biology of Drosophila, v. 2a (Ashburner and Wright, eds.), pp. 1-109; Ringo 1971, DIS 47: 118.

Gilbert, D.G. Indiana University, Bloomington, Indiana. Sperm counts and initial sperm storage in D. melanogaster.

In the course of investigating reproductive functions of the male anterior ejaculatory duct enzyme esterase 6 (Richmond et al. 1980), I have examined the number of sperm initially stored by D. melanogaster females from ejaculates of males differing in their esterase 6 genotype. This note describes the methods used for counting sperm and the major results for 47 matings of 3 to 5 day virgins of the Oregon-R strain.

The dissection methods reported here are modified from those described by Fowler (1973) in two important respects. Female reproductive tracts are dissected directly in aceto-orcein stain rather than in Ringer's saline, avoiding a saline-stain reaction which destroys the specimen within a week. Specimens dissected in the stain and sealed under coverslips preserve for several months. Secondly, the spermathecae are dissected from the uterus, pared of their surrounding fat which inhibits staining, and squashed under a separate coverslip. With this method, sperm heads in the densely packed mass of spermathecal sperm stain deeply enough to count the preparations accurately.

Materials used in dissections are two fine forceps, two tungsten dissecting needles, a dissecting microscope, slides and coverslips, and nail polish for sealing slides. The orcein stain used is the salivary chromosome "dissecting" solution described by Strickberger (1962). Viewing specimens with phase optics at 1000X, the stained 10 micron long sperm heads of D. melanogaster can be readily counted with a hand held counter.

The uterus, with attached ventral receptacle, dorsal spermathecae and parovaria, along with the lower portion of the common oviduct, are simply dissected from the female. A mated, etherized female is placed in a drop of orcein stain on a slide. Squeezing the abdomen with the left forceps, the extruded ovipositor is grasped with the right forceps and pulled posteriorly until the reproductive tract is out of the abdomen. Any exterior chitin and digestive tract are dissected away. To obtain clear counts of spermathecal sperm, these paired organs are dissected from the uterus by severing the spermathecal ducts. The fat is dissected away, and the spermathecae are transferred to a second drop of orcein stain on the slide. After applying coverslips to both spermathecal and uteral preparations, the spermathecae are squashed with a hard pressure that expels the sperm mass entirely from its opaque capsule. The uterus-receptacle is squashed gently to flatten it for phase optics without disrupting receptacle integrity.

Females were dissected at intervals ranging from 10 minutes to 50 hours after mounting of the female by the male, which included interrupted copulations. Details of these results will be reported elsewhere. There is a high degree of individual variation in the sperm storage process; the results reported here are in terms of least squares regression estimates of the population values and individual values. Uteral sperm numbers, when greater than 300, were estimated by measuring the area covered by the uteral sperm mass with an ocular micrometer and counting sperm density at random points in the mass. Receptacle and spermathecal sperm were counted directly. The entire counting time for a specimen ranged from 15 to 45 minutes. Only specimens for which two complete replicate counts could be obtained (N = 47) were analyzed. Counting error (mean coefficient of variation ± SEM) for the combined classes of receptacle, spermathecal and uteral sperm was 2.7 ± 0.91% per individual, after logarithmic transformation of counts. Table 1 lists the maximums and times of maximum sperm numbers for these sperm classes.

Table 1. Initial sperm storage parameters for Oregon-R matings, in terms of regression estimates of population values and individual observed values.

	Maximum number		Hour* of maximum	
Sperm class	Estimated	Observed	Estimated	Observed
Transferred sperm:				
Uteral sperm	5800	4690	0.28	0.25
Stored sperm:				
All organs	1120	1032	5.1	5.3
Receptacle	670	767	4.0	0.9
Spermathecae	390	449	7.0	5.3

*Hour post mounting of female by male.

An important aspect of these results is that the maximum storage of sperm greatly exceeds the commonly referred to value of 750 for maximum storage capacity (Kaplan et al. 1962, based on examination of 8 females). This high amount of storage appears to be balanced by a high degree of sperm waste (loss from the reproductive tract without fertilization) during the first 3 days after mating (Gilbert et al. 1981), causing progeny to stored sperm ratios of approximately 50%. Such an inefficient use of stored sperm, as well as transferred sperm (approximately 10% of transferred may ultimately fertilize eggs), suggests that sperm selection may be an important component of natural selection in D. melanogaster, particularly if sperm genotypes within or between ejaculates differ in their functional abilities.

Supported by NIH AG02035.

References: Fowler 1973, Adv. in Genetics 17:293-360; Gilbert et al. 1981, Evolution, in press; Kaplan et al. 1962, DIS 36:82; Richmond et al. 1980, Science 207:1483-1485; Strickberger 1962, Experiments in Genetics with Drosophila, p. 103, Wiley.

Goncharenko, G.G. and I.K. Zakharov. Institute of Cytology and Genetics, Novosibirsk, USSR. A phosphoglucomutase locus in D. virilis.

Polymorphism at the locus phosphoglucomutase (Pgm) has been extensively studied in may Drosophila species. The genetic localization of this structural locus was determined for some species (Hjorth 1970, Trippa et al. 1970, Lakovaara and Saura 1972, Charlesworth et al. 1977).

The total of 20 lines from seven members of the virilis group of Drosophila (D. virilis, D. americana texana, D. littoralis, D. ezoana, D. novomexicana, D. lummei) was included in this study. The genetic variability of phosphoglucomutase has been studied using starch gel electrophoresis. Each fly was homogenized in 0.025 ml double distilled water on the rough surface of a slide. The starch gel electrophoresis was performed vertically using 12-13% starch and 10% sucrose in medium containing 0.045M TRIS, 0.025M boric acid and 0.001M EDTA. The electrode buffer had 0.18M TRIS, 0.1M boric acid, 0.004 EDTA (anodal) and 0.13M TRIS, 0.07M boric acid, 0.003M EDTA, 10^{-5} NADP (catodal) (Porter et al. 1964). The electrophoresis took 4-5 hours at 5-10°C with a voltage of 320-360v and current intensity of 60-80 ma. Staining mixture as in Ayala et al. (1972).

The electrophoresis of Pgm revealed the presence of three variants, called $Pgm^{0.80}$, $Pgm^{1.00}$ and $Pgm^{1.20}$ on the basis of their mobilities (see Fig. 1). The data from different crosses indicate that these three variants are coded by three codominant alleles at one locus.