Spiess, Luretta D. and Eliot B. Spiess. University of Illinois at Chicago Circle, Chicago, Illinois. Mating frequency and conditions of yeast and water in culture of D. persimilis.

In attempting to stabilize some of the conditions important to mating frequency in this species a three-dimensional classification experiment was run (before the authors moved from Pittsburgh to Chicago). Some possible external conditions leading to variation in mating speed were suspected as follows: 1) tap water (Pittsburgh), 2) type of yeast with various contaminants (Fleischman's Baker yeast cake vs. dry yeast), and 3) presence or absence of live yeast in storage at least 24 hrs. before mating tests. Culture medium was corn-meal-molasses-yeast-agar-propionic acid-tegosept.

Flies from the 27th generation of our fast development selected population (DF population in Spiess and Spiess, Genetics 50: 863-877, 1964) were isolated, determined to be Whitney (WT) homokaryotypes, and mass mated in bottles provided with plastic spoons containing food and yeast according to four combinations: using either tap water or distilled and using either dry yeast or yeast cake. (See Spiess and Langer, Evolution 18: 430-444, 1964, for previous methods.) Spoons with 100 - 200 eggs were inserted into the same type of food in bottles and cultured at 15°C. On emergence, adults were sexed and isolated in vials containing the same type of food for 7 days, at which time they were transferred to fresh vials in lots of 10; these vials were then either provided with the same type of yeast or not given any yeast for 24 hrs. Mating frequencies (60 minutes' observations) are given in the following table together with an analysis of variance. One hundred pairs were tested at each combination.

Percentage Matings (DF Population Progeny)

	Storage Conditions	
Culture Conditions	With live yeast	Without yeast
Tap water		
+Dry yeast:	82%	59%
+Cake yeast:	69%	41%
Distilled water		
+Dry yeast:	85%	41%
+Cake yeast:	80%	50%

Analysis of Variance for Mating Data
(80 chambers of 10 pairs per chamber)

	d.f.	Sum Sq's.	M.S.	F
Between treatments:	7	234.3	----	(n.s. = not significant) (** = p<.01 * = p<.05)
Between:				
Tap-distilled	1	0.6	----	n.s.
Dry-cake:	1	7.8	7.8	2.11 n.s.
Yeast in storage (+) - (-):	1	201.6	201.6	54.34**
Interactions	4			
Water x Yeast	1	13.6	13.6	3.67*
Yeast x Storage	1	1.5	----	n.s.
Storage x Water	1	5.5	----	n.s.
Storage x Water x Yeast	1	3.7	----	n.s.
Within treatments:	72	266.9	3.7	n.s.
Total:	79	501.2		

It can be seen that live yeast at least 24 hrs. before mating is critical to the outcome. Probably the live yeast grows better in distilled water than in the tap so that the water x yeast interaction has borderline significance. It is likely that conditioned by

live yeast in nature at the food source to mate, and it is essential to provide them with live yeast in studying mating behavior. This fact has been mentioned by Manning and others in the past, but no data have been published heretofore on this point.
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Lifschytz, E., and R. Falk. The Hebrew University, Jerusalem, Israel. A new system for fine structure analysis of genes in Drosophila.

In recent years a number of systems for high power resolution of recombination and back-mutation have been described for Drosophila. We constructed a system for high power resolution analysis based on the lethal interaction between pn and K-pn and on lethals in the X-chromosome, covered by segments of the X-chromosome translocated to the Y-chromosome. The stages that were involved in the construction of the system will be described elsewhere. Only the final system is given here:

a. $\frac{y^2\ pn^i\ cv\ l^{B57}}{F\ M\ 6\ (1)} \times \frac{pn^j\ l^{3des}}{Y\cdot\ pn^-w^+}$

b. $\frac{y^2\ pn^i\ cv\ l^{B57} + \quad +}{pn^j \quad + l^{3des}};\frac{}{+} \times \frac{v\ g\ l^{B57}\ l^{3des}}{Y\cdot\ ma\text{-}l^+};\frac{ca\ K\text{-}pn}{ca\ K\text{-}pn}$

The system includes two stages:
a. The stage for large scale "automatic" collection of virgin females.
b. The stage for the detection of recombinants in heterozygotes for different pn-alleles or of back-mutations in homozygotes for a single pn-allele.

It is easy to see that all progeny should die, excluding males that are recombinants (or back-mutants) in the pn-locus and females that are recombinants between the lethals or products of non-disjunction in the fathers.

For the "automatic virgin" system the lethals were chosen so that one was proximal enough to be covered by both $w^+\cdot Y$ and by $ma\text{-}l^+\cdot Y$, while the other was somewhat more distal, thus covered only by the $ma\text{-}l^+\cdot Y$-chromosome. We verified that the frequency of non-disjunction was low and that the lethals did not produce "Durchbrenners" even under the uncrowded conditions that prevailed when most larvae died. The lethals were chosen so that there was about 0.5% crossing-over between them, so that there were enough viable progeny per culture (3-10 females) to facilitate the eventual single rare recombinant between pn-alleles. Furthermore, since the frequency of recombination between the lethals was predetermined, the number of females per culture served to estimate the total number of zygotes that had been produced.

In order to minimize the work involved, we found it best to use 20 pairs of flies per mating in 1/4 litre culture bottles (somewhat above the optimum) and to transfer the parents twice to fresh cultures, after they stayed for 4 days in the old ones. Only a sample of the bottles was etherized and from them the mean number of females per culture was determined. In the remaining bottles the presence of males was checked by inspection without etherization. Analyses of 1.2×10^6 zygotes may be carried out routinely by a single technician.

Preliminary analyses resolved the pn-locus into three sites: $pn^1\text{-}pn^2, pn^{59j}\text{-}pn^{AA1}$.

A somewhat modified system in which the recombinants between pn-alleles were the female progeny and the recombinants between the lethals were the male progeny was also constructed. With proper selection of lethals these systems may be utilized also for analyses of other events in the chromosome, such as unequal crossing-over or negative interference.