THE ISOLATION AND CHARACTERIZATION OF BEHAVIOURAL MUTANTS OF DROSOPHILA MELANOGASTER

By

JOHN KENT SARGEANT, B.Sc.

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AUTHOR: John Kent Sargeant, B.Sc. (McMaster University)

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ABSTRACT

Using the mutagen ethyl methanesulfonate, strains of Drosophila melanogaster were isolated whose males displayed mutant mating behaviour. Three lines were selected for closer study. One, strain #55, was associated with a morphological mutation that decreased male courtship success. Of the two non-morphological mutants, one strain (#263) exhibited almost no mating activity under test conditions, while the other (#277) was more successful than standard Canton-S males. Through mapping and test crosses, the mode of inheritance of each mutant was investigated. Other behavioural tests were performed to assess locomotor activity. These three mutants are discussed in terms of current ideas regarding courtship in Drosophila.

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Introduction

The establishment of behavioural genetics as a legitimate branch of science is commonly held to have occurred with the publication of Behaviour Genetics by J.L. Fuller and W.R. Thompson in 1960. Despite its relatively young age in a technical sense, behavioural genetics has origins predating 1960, and this is for two reasons. First, Fuller and Thompson's book represented a consolidation of the then current ideas and consequently drew on experimental work done in the 1940's and 1950's. Secondly, and more importantly, behavioural genetics is by nature a synthetic science - born from the older disciplines of ethology, psychology and genetics (Ehrman and Parsons, 1976).

It is because of this multi-disciplinary origin that the fundamental problems in behavioural genetics are to a large degree but reformulations of previous questions. Historically one of the most enduring of these has been the "nature versus nurture" debate. Stemming from the philosophical tension between extreme environmentalism and (genetic) determinism, the

conflict continued in behavioural terms via the learning/conditioning school (represented by B.F. Skinner and his associates) on the one hand, and the innate/instinctive school (exemplified by Konrad Lorentz) on the other.

Presently the debate has subsided with the realization that the former options were over-simplified and unduly polarized, and that there is in fact a continuous spectrum between the two extremes. In addition, the poles themselves are now regarded as being non-existent abstractions. Even the deepest innate responses are contingent upon basic exchanges with the environment for their developments and the ability to learn or be conditioned is restricted by the genetic heritage of a species. (Alcock, 1975)

In this sense, what was once a major goal of behavioural genetics - i.e., the proof of a genetic role in different behaviour patterns - has become superfluous. Having thus become released from the need for simple demonstrations, questions regarding physiological and causal relationships between genes and behaviour and quantitative analysis of behavioural variation as it reflects genetic variation could be approached.

A fundamental orientation of behavioural genetics at all times has been the treatment of behaviour as a phenotype (Manning, 1965; Broadhurst and Jinks, 1974). Regarded in this light, it becomes both a product of and subject to the same

forces that drive the more familiar morphological evolution. The behavioural phenotype under consideration here is male courtship in Drosophila melanogaster.

As described by Sturtevant (1915), catalogued by Spieth (1952), and quantitatively assessed by Bastock (1956) and others, the events leading to copulation in D. melanogaster involved the following sequence:

1. The male fly encounters a female and taps her with his foretarsi, usually on her abdomen.

2. The male orients himself behind and to one side of the female, facing her about 2-3mm away.

3. With his abdomen curled under and forward, the male engages in wing display wherein one wing (usually the wing closest to the head of the female) is extended 90° in the horizontal plane and vibrated rapidly for .5-2 sec.

4. During and/or between bouts of wing vibration, the male extends his proboscis to contact the vaginal plates of the female. This activity is commonly known as licking.

5. Receptive females spread their vaginal plates. The male curls his abdomen further and lunges forward, establishing genital contact. The male then mounts the female farther forward by spreading her wings with his head and forelegs. Copulation lasts for approximately 15 minutes in D. melanogaster (MacVean and Parsons, 1966).

The initiation of courtship by a male does not insure a successful conclusion. At all times prior to genital contact, courtship may be terminated by either participant. The female, for example, may give one of a number of counter-signalling responses to a courting male (by retracting, by depressing or elevating the abdomen, or by extrusion of ovipositor). The male will usually not perform the above steps in rigid sequence, rather courtship includes repeated bouts of the first four steps in varying order.

Despite this variability in the exact performance of male courtship, behavioural patterns for displays are species-specific in terms of the initial order in which the components occur. Species specificity is also reflected in the repertoire of acts - some species lacking a vibration component, and others having retained it only during the copulatory phase itself (Spieth, 1952; Manning, 1965). Because of this specificity and because male flies raised in isolation are able to successfully court and mate with females in their first sexual encounter, courtship in Drosophila is regarded as being at the "innate" extreme of the behavioural nature-nurture scale. (Burnett and Connolly, 1974).

The description of male courtship above is largely a "phenomenological" one. Identifying the precise function and significance of both male and female activities during a sexual

encounter requires a greater degree of interpretation. One reason for this is the considerable amount of variation that a behavioural act exhibits, variation that undoubtedly arises from its being contingent upon a multitude of subtle and complex cues for its generation. For those interested in Drosophila mating behaviour, this is further complicated by the fact that one is concerned not only with the behaviour of a single individual but with a pair. Consequently one must contend with male behaviour and female behaviour together with all the interactions that occur between them.

Despite this multifaceted complexity, the study of the behaviour genetics of courtship certainly is concerned with the functional aspects of courting activity. Current views rest heavily on the idea of Darwinian fitness and how it may be maximized in Drosophila. For males, fitness is highly correlated with mating speed and the number of females inseminated (Fulker, 1966), hence it is expected that selection has maximized mating speed and compromised discriminative ability (Speiss, 1970). Males will approach any and all females to initiate courtship. Since a male also will investigate other males, as well as foreign objects with the same general shape of a female, it seems that the primary initial stimulus to the male is received visually. The observation that visual discrimination in Drosophila is crude explains

the highly tentative nature of the male's preliminary advances. For courtship to continue, he must receive the proper counter-signals from the female. These are not well understood and may have a chemosensory or olfactory basis. This information may be relevant to species recognition as well as stimulating the male into the succeeding courtship display itself. The component actions are repeatedly performed and not until the female signals acceptance is the male thought to attempt genital contact. The acceptance signal is usually the spreading of the vaginal plates, although in some species the female parts her wings as well (Spieth, 1952). Since males are indiscriminate courters and courtship initiation is apparently based on visual stimuli, males are viewed as having a low threshold of stimulation for engaging in courtship and all of this as a result of selection for fast mating speed.

The discriminating ability of the female is well documented (Petit and Ehrman, 1969; Speiss, 1970 for reviews), and contrasts with the male's lack of mating preference. Both sexes possess sexual isolating behaviours but only the female seems to exert the force of sexual selection. Although in the wild a high percentage of females are inseminated (~90%), they mate relatively infrequently and are able to store sperm from a single mating for a period of days (Spieth, 1952).

The basis of the female's discriminative ability is not

fully understood. Their role as agents of sexual selection has been presumed to be directed in some way at male "vigor" or "athletic ability" (Petit and Ehrman (1969), p.204), admittedly vague notions. However, the vibration component correlates well with male mating success (Bastock, 1956) and is probably of major stimulating value to the female. Whether this is achieved through olfactory or auditory means is not known.

If present knowledge of what the female responds to is couched in "ill-defined yet meaningful qualities" (Speiss, 1970), the means by which she receives relevant information is somewhat better understood; female antennae are known to be important in maintaining sexual isolation (Mayr, 1950; Ehrman, 1959; Manning, 1967) and function in their sexual selective role as well (Begg and Packman, 1951).

The difficulty in evaluating exactly what stimuli the female detects and responds to as well as how she receives this information may be due to the subtlety and interactive nature of the relevant cues. As Spieth has noted (1952), heterogenous stimuli are probably required for adequate female stimulation. Moreover, it appears that "what is necessary is that the heterogenous stimuli added together are sufficient to reach the threshold of reaction of the individual being studied" (Spieth, 1952, p.462).

The present model of Drosophila sexual behaviour, then,

postulates males as having a low threshold for courtship initiation which confers a constant readiness to mate, and females with a higher threshold of acceptance which is reached by heterogenous summation of stimuli. This summation process occurs as a function of those attributes of male courtship towards which her discriminative powers are directed.

Inherited species-specific courtship behaviours offer useful systems for studying the genetics of a behavioural phenotype, and this is all the more true for Drosophila melanogaster which has been well characterized both behaviourally and genetically (Burnett and Connolly, 1974). There are two possible approaches to this end. The biometrical methods (Jinks and Broadhurst, 1974; Mather and Jinks, 1971) are directed to problems in quantitative character. Through diallel cross analysis, information about whole chromosome or genome effects on mating behaviour has been gained (Parsons, 1964; Fulker, 1966).

In contrast to biometric analysis, the Mendelian approach concentrates on simple genetic systems with one to a few loci. Because of this methodological orientation, the behavioural effects of each locus must be great enough to make discrimination among genotypes possible, given the large amount of behavioural variation within lines. Most studies of this type have been concerned with exploring pleiotropic

effects of known morphological mutants on sexual behaviour (see Grossfield, 1975 for a review). More recently, practical screening methods have been devised to assay directly for altered non-sexual behaviour (Ikeda and Kaplan, 1970; Konopka and Benzer, 1971; Pak et al, 1969; Pak, 1975 review).

The use of single gene changes as tools for dissecting behaviour patterns is especially well suited to studying associated physiological processes. Moreover, more powerful genetic techniques are able to be used to elucidate functional relations between genes and behaviour (genetic and mosaic mapping, for example). Consequently, it was decided that the Mendelian approach would be used to study mating behaviour in D. melanogaster. Specifically, this took the form of generating mutant lines with ethyl methanesulfonate (EMS) and selecting for males with altered mating behaviour. Since it is known that females can discriminate among males, the rationale was to present genetically different males to females of a standard stock and to compare mating activities among the males. Defective behaviour was of primary interest because, as a complex behaviour pattern dependent upon the proper functioning of a multitude of systems in the fly, random changes in any of them would likely be detrimental to overall courtship performance. Moreover, it has already been indicated that natural selection has tended to maximize male mating

speed in Drosophila (Manning, 1961). Again, any deviation would probably be in the direction of decreased mating speed.

There are three general classes that mutants may fall into using this kind of scheme. The less interesting cases involve changes in external morphology such that effects on mating behaviour result. The mutant antennaless is an example of this type. The loss of functional impairment of so important a sensory organ (Barrows, 1907; Waterman, 1950) is fully expected to (and does) affect the behaviour of the fly in many ways, including sexual behaviour (Mayr, 1950; Manning, 1967a; Bagg and Packman, 1951). General behaviour mutants, on the other hand, are non-morphological with respect to external anatomy. Internal lesions in muscular, neural or other systems, however, can give rise to nonspecific behavioural changes. Locomotory mutations, for example, may affect mating and oviposition behaviour as well. The third class is restricted to those behavioural mutants where systems or functions that are used only in sexual interactions are affected. Because of the complex involvement of many systems in the generation of all behavioural acts, these are expected to be in the minority.

The experiments described here were based on these considerations. By using the relative behavioural rigidity of male Drosophila courtship as a paradigm and the genetic simplicity

of a Mendelian approach, single gene mutants for mating behaviour were isolated and studied.

Materials and Methods

1. Stocks and Culturing Methods

All strains used in these experiments were of the species Drosophila melanogaster. The laboratory standard Canton-Special and attached-X populations (hereafter referred to as CS and $\overline{XX}$, respectively) were kindly provided by the California Institute of Technology. The detailed genetic constitution of $\overline{XX}$ stock is given in Lindsley and Grell (1968), but the relevant features are as follows: Females of this strain possess two X-chromosomes (attached, and both carrying recessive markers yellow (y^2), white eye (w), and forked bristles (f)), plus a Y-chromosome, while males (XY) carry only a recessive sex-linked temperature sensitive lethal mutation. Genetic mapping of mutants generated in the course of this study was done using strain no.206 from Caltech, which carries recessive sex-linked markers yellow (y^2), crossveinless (cv), vermillion (v), and forked (f).

All cultures were raised on Carpenter's medium (see Appendix 1) and were cleared daily. The sexes were separated using carbon dioxide anesthetization whereby CO_2 was forced

into a vial of newly eclosed flies until they were immobile (.5-1 sec.). Separation of the sexes with a paintbrush was done on a cooled plastic Petri dish which was inverted on top of crushed ice. This technique has many important advantages for behavioural studies with Drosophila since there is no danger of toxic or fatal over-exposure that occurs with the more common etherizing procedure. Moreover, flies remain unconscious as long as they are kept cool - hence the need for the Petri dish and ice - without further ill-effects. The extreme rapidity with which CO_2 renders flies inactive is an added practical benefit of this method.

Samples thus collected were retained in disposable plastic tubes (17 x 100mm) which contained 2-3 ml. of Carpenter's medium. All flies were aged for four days prior to testing, to standardize sexual maturity and receptivity (Manning, 1967). All tests were conducted between 2pm and 6:30pm.

2. Mutagenizing Procedure

Mutant stocks were generated through the use of the mutagen ethyl methanesulfonate (EMS) following the procedure of Lewis and Bacher (1968). Fifty to one hundred newly eclosed (~24 hours old) CS males were starved for 24 hours. The starved males were then placed in a jar containing four

Kimwipes that had been previously soaked with approximately 4 ml. of EMS-sucrose solution (see Appendix 2). The flies were allowed to feed for 24 hours after which time they were mass-mated to 200 $\overline{XX}$ virgin females. Using the rationale of Benzer (1967) and Pak (1975), male progeny from this mass mating (designated F1) were mated singly to $\overline{XX}$ virgin females to produce lines whose males shared a possibly mutant X-chromosome derived from a CS male, while the females were maintained in their attached-X condition. Each mated pair (FI ♂ x $\overline{XX}$ ♀) thus gave rise to a numbered line which was cultured inter se (see Figure 1).

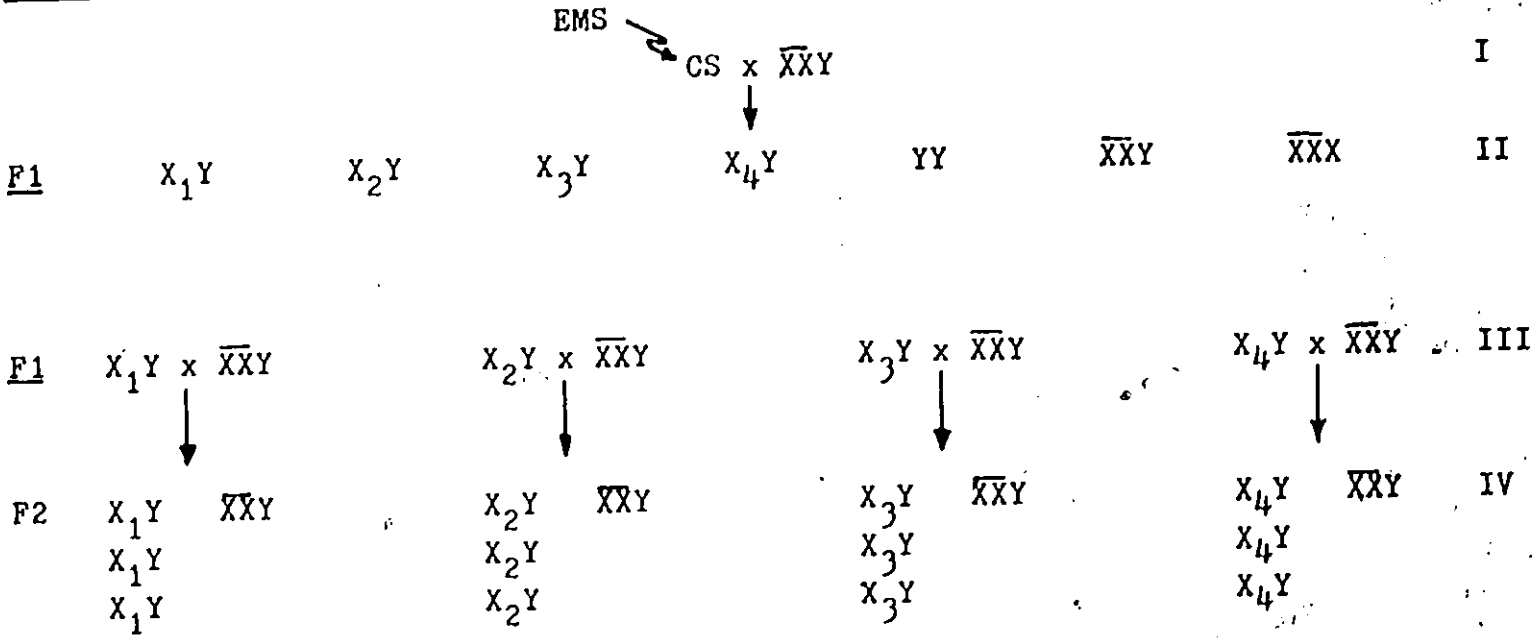
Lines were chosen for closer study on the basis of the behaviour of the F1 male parent of a given line. Progeny of those not mating within a 30-minute observation period were used in further investigations of mating activity both in the light and in darkness.

3. Mating Test Procedures

a) Tests Conducted Under Illuminated Conditions:

Males and virgin females ($\overline{XX}$ virgins, unless otherwise noted; both sexes were four days old) to be tested were placed singly without anesthetization into 10 x 70mm glass test tubes that contained approximately 1 ml. of Carpenter's medium. This was achieved by tapping flies gently from the 17 x 100mm holding tubes into empty 10 x 70mm test tubes via

Figure 1. Mutagenizing Crosses



Note: I) mass mating of EMS-fed CS males to $\overline{X}\overline{X}Y$ virgins

II) only viable offspring are $\overline{X}\overline{X}Y$ daughters, and XY (F1) males (each a possible carrier of a unique X-linked mutation)

III) F1 male behaviour recorded in single pair matings; those not mating within 30 minutes were noted and allowed to mate for extended time

IV) progeny of 'non-maters' cultured inter se; males tested for mating activity.

a polypropylene funnel, and then allowing single flies to crawl into inverted 10 x 70mm tubes. Tubes containing single flies were sealed with clean sponge tops and grouped according to sex and strain. Once all the flies to be tested in a given experiment were isolated in this manner, male flies were tapped gently into a tube containing a female. The tube containing the pair was stoppered with a sponge top and laid on its side on the laboratory benchtop for observation. Numbered pairs of flies were set sequentially and the time to the onset of copulation for each pair was recorded to the nearest five minutes. The large number of pairs in a group to be tested precluded the observation and timing of each pair individually.

Because about 5 sec. elapsed between the setting of one pair and the next, the total time for the placement of a particular group being tested was recorded in stopwatch. This information was used to modify the raw observations to more accurately reflect the real time interval between the introduction of a male into a tube containing a female and the beginning of copulation (see Methods, Part 6 (Data Analysis) for further discussion).

All $\overline{XX}$ virgins used in both light and dark tests were from the original $\overline{XX}$ stock and not from the derived mutant

lines. Pairs were observed for a maximum of 35 minutes. Except where otherwise noted, a minimum of 100 pairs per strain were tested in sets of not less than 10 pairs for a given experiment. Mating tests were conducted at 25 $\pm$ 1.5° C. Illumination was measured at 2.3 $\mu E\ m^{-2}\ sec^{-1}$.

b) Tests Conducted Under Dark Conditions:

Three-day old male and female flies were dark-adapted for 24 hours at 25°C. They were then placed singly into 10 x 70mm test tubes containing approximately 1 ml. of Carpenter's medium without anesthetization as described in part (a) above. Since it was necessary to isolate the flies under minimum lighting conditions, a 25-watt incandescent lamp was used in conjunction with a fitted dark red filter. The resultant illumination was .01 - .1 $\mu E\ m^{-2}\ sec^{-1}$ in the dark room working area during both fly separation and while pairs were scored for copulations. As mentioned previously, males were transferred into tubes containing a female. Pairs of flies were placed immediately into hinged opaque plastic boxes to eliminate any residual light cues.

Unlike the experiments performed in the light, these dark tests did not involve repeated observations taken at different times, rather they were composed of a single observation for each group. It was therefore necessary to choose an appropriate period of time that would maximize the number of copulations

yet minimize the number of (unscored) completed matings. The average duration of copulation in Drosophila is 13-20 minutes (MacBean and Parsons, 1966). Consequently, 15 minutes was chosen as an incubation time, after which the tubes were sequentially removed from the plastic boxes and scored for copulatory activity.

4. Locomotor Activity Tests

Sixty to seventy four-day-old males were starved for one hour and then placed in the terminal tube of a sixth order countercurrent apparatus (see Figure 2). Flies were allowed to equilibrate for one minute. The apparatus was kept horizontal with one row of tubes facing two 15-watt fluorescent lamps 10cm away. The operation of the countercurrent apparatus was as in Benzer (1967), with a cycle time of 30 seconds. Clean, clear disposable plastic tubes (17 x 100mm) were used for each experiment and the final distribution of flies in the six tubes was recorded. Extraneous visual cues were screened by placing the apparatus in a three-sided open top box (Figure 2). Illumination in proximal and distal ends of the tubes was 39 and 10 μE m^{-2} sec^{-1} respectively.

5. Data Analysis

a) Mating Tests (Illuminated):

The large number of test pairs in a given experiment

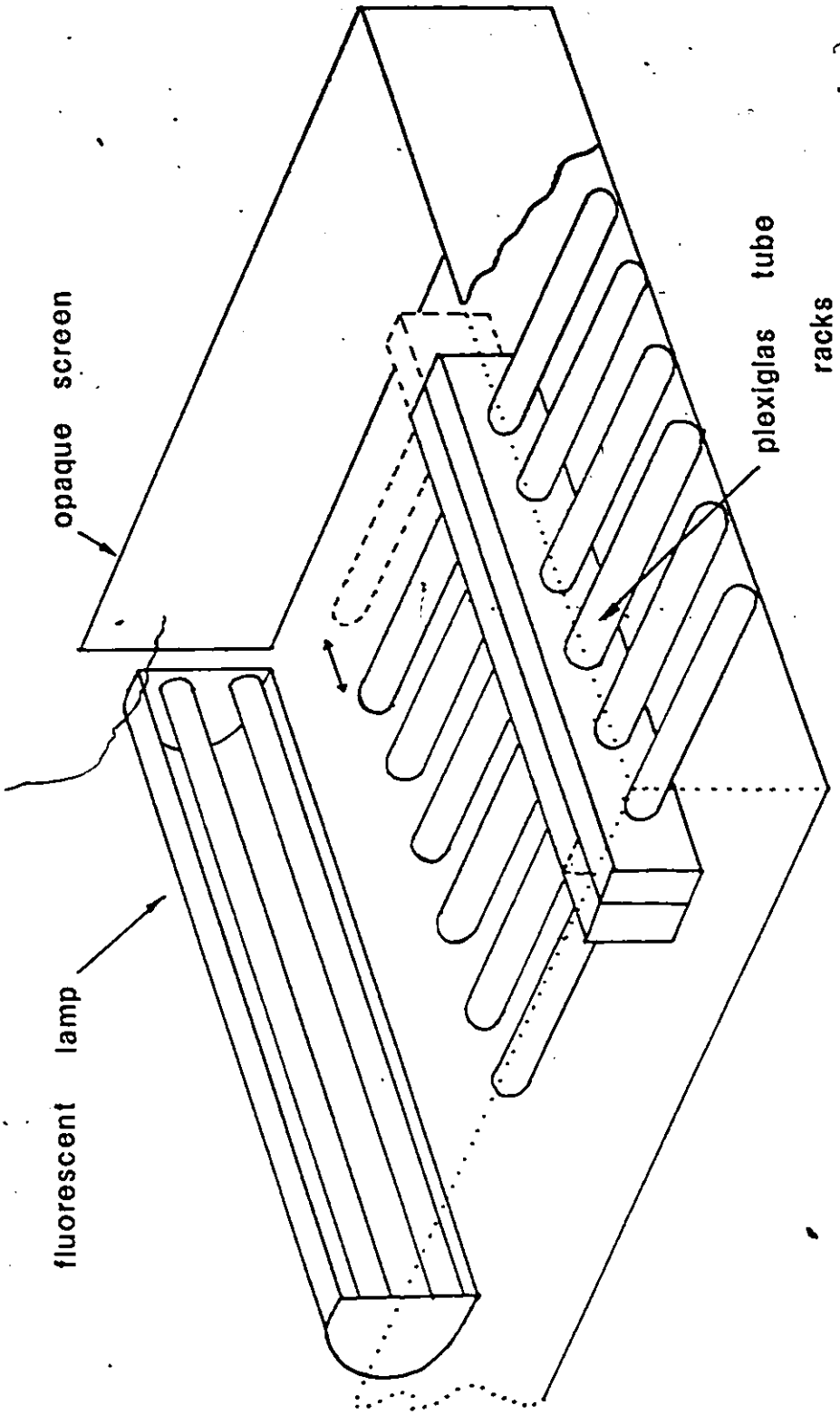


Figure 2. Countercurrent Apparatus

made the continuous observation of each pair impossible. Initially data were recorded as the time from the introduction of a male fly into a tube containing a female to the time of the onset of copulation. Specifically, each tube was scored at the end of 5-minute intervals for the presence or absence of copulatory activity. The placement of the first male with a female was designated as time = 0. Thus, for example, if pair number 3 began copulation at 6' 3", they were recorded as having mated between 5 and 10 minutes.

As mentioned in section 3 (a) above, the finite time required to set test pairs would result in some distortion of the data. For example, if 50 pairs were to be tested, approximately 4 minutes would elapse for the setting of pairs 1 through 50. At the end of the first 5-minute interval, the last pairs to be set would have had only 1 or 2 minutes to mate while the first ones would have had about 5 minutes. Furthermore, if the last pairs did mate after 2-3 minutes of incubation, they would be recorded as having mated between 5 and 10 minutes. Consequently, the original observations were scaled in the following manner:

Having recorded the total time for setting all pairs in a given experiment as well as the number of pairs tested, it was possible to determine when particular pairs were set. The rate of setting pairs varied from 8 pairs/min. to 10 pairs/min.

for different experiments. For simplicity, 2.5 and 7.5 minutes were chosen as demarcation times such that pairs set at time $2.5 < t < 7.5$ and $7.5 < t$ were reassigned to the 5 or 10 minute interval, respectively, preceding the one in which they were originally noted as having mated. As a result, if a pair began copulation between 10 and 15 minutes, it was placed in:

a) the 10-15 min. mating class if it was set before 2.5 min.;

b) the 5-10 min. mating class if it was set before 7.5 min. (but after 2.5 min.);

c) the 0-5 min. mating class if it was set after 7.5 min.

(Note that all times were measured relative to the setting of the first pair.)

Mathematically, the original observations had no limit to the error difference (that is, the recorded mating class minus the true mating class), but this was dependent upon the number of tubes set. The modification described here did not eliminate all inconsistencies. (For example, if a pair was set at $t_1 = 3$ minutes and copulation began at $t_2 = 9.5$ minutes, corrected data would list this pair in the 0-5 minute class whereas $t_2 - t_1 = 6.5$ minutes had actually elapsed.) Nevertheless, since the data were recorded to the nearest 5 minutes this technique was appropriate to the level of accuracy used.

The modified data were used to construct cumulative frequency distributions of matings through time, both relative to the total number of pairs tested (the number of attempts) and the total number of pairs that actually mated. Because observations were summed over all samples, final distributions represented weighted means of samples for the strains tested (see Figure 3 (i) as an example).

The Kolmogorov-Smirnov two sample test was used to determine whether significant differences between cumulative distributions existed. The non-parametric nature of the test allowed the relaxation of the assumption of normality while retaining good power (Sokal and Rohlf, 1969). The relevant statistic (D) measures the probability of finding a difference as extreme as (or more extreme than) the observed maximum difference between two continuous distribution functions. Since all tests used sample sizes on the order of 100 pairs, the large sample approximation was used. The D statistic then becomes a function of sample sizes (n_1, n_2), such that in general the critical difference, D, equals $\lambda\sqrt{\frac{n_1+n_2}{n_1 n_2}}$. The coefficient λ is a constant for each level of significance (Beyer, 1966). Since comparisons were made only between two curves at a time, confidence intervals for cumulative distributions are not depicted in the graphical figures. Critical values for the Kolmogorov-Smirnov statistic are tabulated in

Appendix 3 for all comparisons.

b) Mating Tests (In Darkness):

The results from experiments on mating activity under the dark conditions were composed of one proportion for each strain (i.e., the fraction of the test population that mated). As with the mating tests conducted in the light, these were summed so that they represented weighted means of the samples tested. The G-test of independence was used, based on the method of Sokal and Rohlf (1969), rather than the chi-square test because of the relative ease of calculations and because more detailed analysis was possible. This was used to see whether the frequency of copulations after 15 minutes incubation in the dark was independent (or dependent) of the strain of fly under test.

c) Locomotor Tests:

In general, the conditions of the locomotor test described in Methods, part 4, led to a six-celled distribution of flies that was skewed to the left. To detect differences in these distributions, the G-test of independence was employed. The multiway analysis permitted testing for the homogeneity of replicates as well as for the independence of strain versus distribution.

d) In some cases, additional analyses of data were

performed. These special topics are discussed as they occur.

Results

1. Mutant Isolation

Completion of mating by an F1 male during the 30 minute time period of the mating test disqualified it and its potential progeny from further consideration. This initial screening eliminated approximately 69% of those tested. Logically, those not mating during that time were not necessarily behaviourally mutant since 20-25% of CS control males did not mate during the same time period; nevertheless, the non-mating group was of prime interest.

Male progeny from the non-mating F1 males were retested in subsequent generations. As testing proceeded some lines did not exhibit abnormal mating frequencies and were discarded after 60-70 mating pairs had been observed. Others demonstrated consistent statistically significant deviations from the rest (especially from the CS control males), and testing continued until 100-150 pairs per strain had been observed. Some of the uninteresting lines were retrained for mating tests for comparisons.

The results of these first population tests are given

Figure 3. Mating Activity (Light):
Initial Data for Various Strains

The results of experiments on mating activity in the light are summarized in similar tables for all tests (figs. 3, 8a, 10a, 13a, 16a). Each table presents results for one strain or subpopulation of males tested against a standard female type. The males and females used are signified at the top of each table; the left and right designations referring to the male and female types, respectively. (Thus, figure 3(i) presents data from experiments on CS males and $\overline{XX}$ females.) Table entries represent the number of males observed to begin copulation during a five minute interval. For brevity and simplicity, column headings are given for the end of the corresponding period. Column totals were divided by the total number of males tested and by the total number of observed matings to give cumulative distributions 'of attempts' and 'of copulations', respectively. These distributions thus distinguished between the total number of males tested and the total number of actual copulations that were observed.

Fig. 3 (cont'd)

i) CS - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	15	1	2	1				19	19
2	7	8	12	3	2	1		33	54
3	4	3		1				8	26
4	7	3		4	1	1		16	24
5	7	2	1					10	13
6	12	2	1	2		1		18	19
Total	52	19	16	11	3	3	0	104	155
Cumulative frequencies (%)									
	33.5	45.8	56.1	63.2	65.2	67.1	67.1	(attempts)	
	50.0	68.3	83.7	94.2	97.1	100	100	(copulations)	

ii) 45 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	1	1	2	2	3			9	16
2	2	2	3		4			11	12
3	9	4	2	3	3			21	24
4	12	4	2	2				20	23
5	7	2	2	3				14	16
Total	31	13	11	10	10	0	0	75	91
Cumulative frequencies (%)									
	34.1	48.4	60.4	71.4	82.4	82.4	82.4	(attempts)	
	41.3	58.7	73.3	86.7	100	100	100	(copulations)	

Fig. 3 (cont'd)

iii) 55 - $\bar{X}\bar{X}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1		4	3	1				8	16
2	3	2	2		2			9	19
3		2			1			3	12
4	1	1		1	1			4	24
5	1	1		4	4	6	5	21	47
6	2	1		1		2		6	10
7			2					2	12
8	2	1	2					5	12
Total	9	12	9	7	8	8	5	58	152

Cumulative frequencies (%)

5.9	13.8	19.7	24.3	29.6	34.9	38.2	(attempts)
15.5	36.2	51.7	63.8	77.6	91.4	100	(copulations)

Fig. 3 (cont'd)

iv) 250 - $\overline{XX}$

Run	Time (min.) 5	10	15	20	25	30	35	Total mated	Total tested
1	5	3		2	1			11	20
2	3	6	2	1	2	2		16	22
3	8	3		1	1			13	13
4	6	3	1					10	13
5	12	3	1			1		17	19
6	3	3	6	1		1		14	16
7	6	3	1					10	12
8	1	2	4	2				9	9
Total	44	26	15	7	4	4	0	100	124

Cumulative frequencies (%)

35.5	56.5	68.5	74.2	77.4	80.6	80.6	(attempts)
44.0	70.0	85.0	92.0	96.0	100	100	(copulations)

Fig. 3 (cont'd)

v) 263 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1								0	12
2								0	10
3			1					1	10
4								0	12
5		1						1	12
6								0	25
7								0	19
8								0	44
Total	0	1	1	0	0	0	0	2	144
	Cumulative frequencies (%)								
	0.0	0.7	1.4	1.4	1.4	1.4	1.4	(attempts)	
	0.0	50	100	100	100	100	100	(copulations)	

Fig. 3 (cont'd)

vi) 271 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	10				2			12	12
2	10	3	1	1				15	16
3	8		1	1				10	15
4	7	4	1			1		13	17
5	2	8	1	4				15	16
6	9	4	1					14	23
7	5	3	1	1				10	12
8	2	2	1	2		2		9	10
9	6	1	2					9	14
10	2	2	2	1				7	8
Total	61	27	11	10	2	3	0	114	143

Cumulative frequencies (%)

42.7	61.5	69.2	76.2	77.6	79.7	79.7	(attempts)
53.5	77.2	86.8	95.6	97.4	100	100	(copulations)

Fig. 3 (cont'd)

vii) 274 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	6	3	1	1				11	16
2	1	4	2					7	9
3	4	1	1		1			7	8
4	4	1	1		1		1	8	11
5	5	2	1	2	1			11	15
6	13	2	1					16	23
7	8	2	1					11	13
Total	41	15	8	3	3	0	1	71	95

Cumulative frequencies (%)

43.2	58.9	67.4	70.5	73.7	73.7	73.7	(attempts)
57.7	78.9	90.1	94.4	98.6	98.6	100	(copulations)

Fig. 3 (cont'd)

viii) 277 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	9	1			1			11	12
2	10					1		11	13
3	7	1	1	1	1			11	11
4	9	1						10	10
5	10	5				1	1	17	17
6	6	1		1	1			9	9
7	7		1					8	9
8	3	3	1					7	8
9		6	1	1				8	8
10	5	1	1					7	8
Total	66	19	5	3	3	2	1	99	105

Cumulative frequencies (%)

62.9	81.0	85.7	88.6	91.4	93.3	94.3	(attempts)
66.7	85.9	90.9	93.9	97.0	99.0	100	(copulations)

Figure 4a. Cumulative Distributions: Initial Data

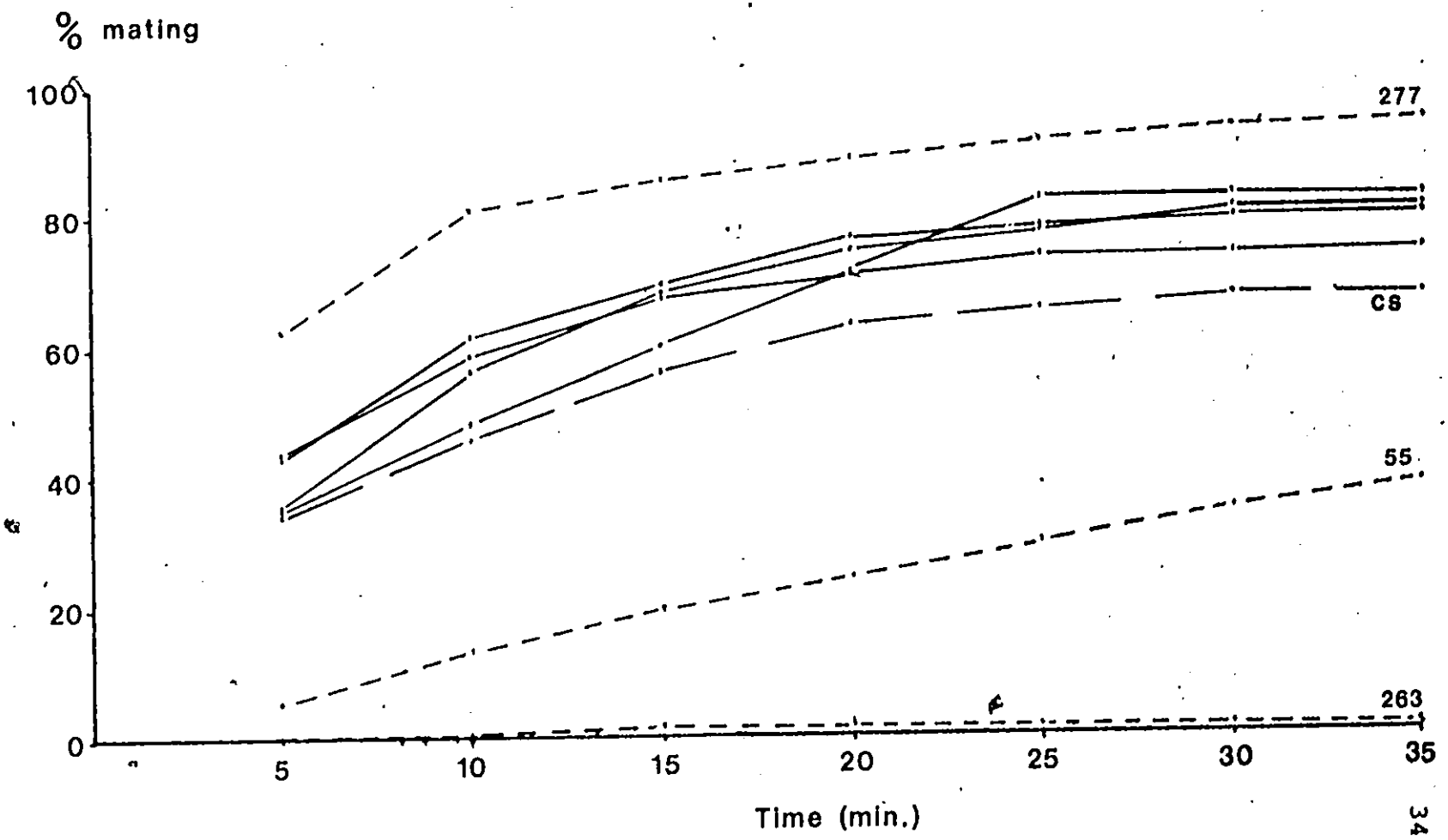
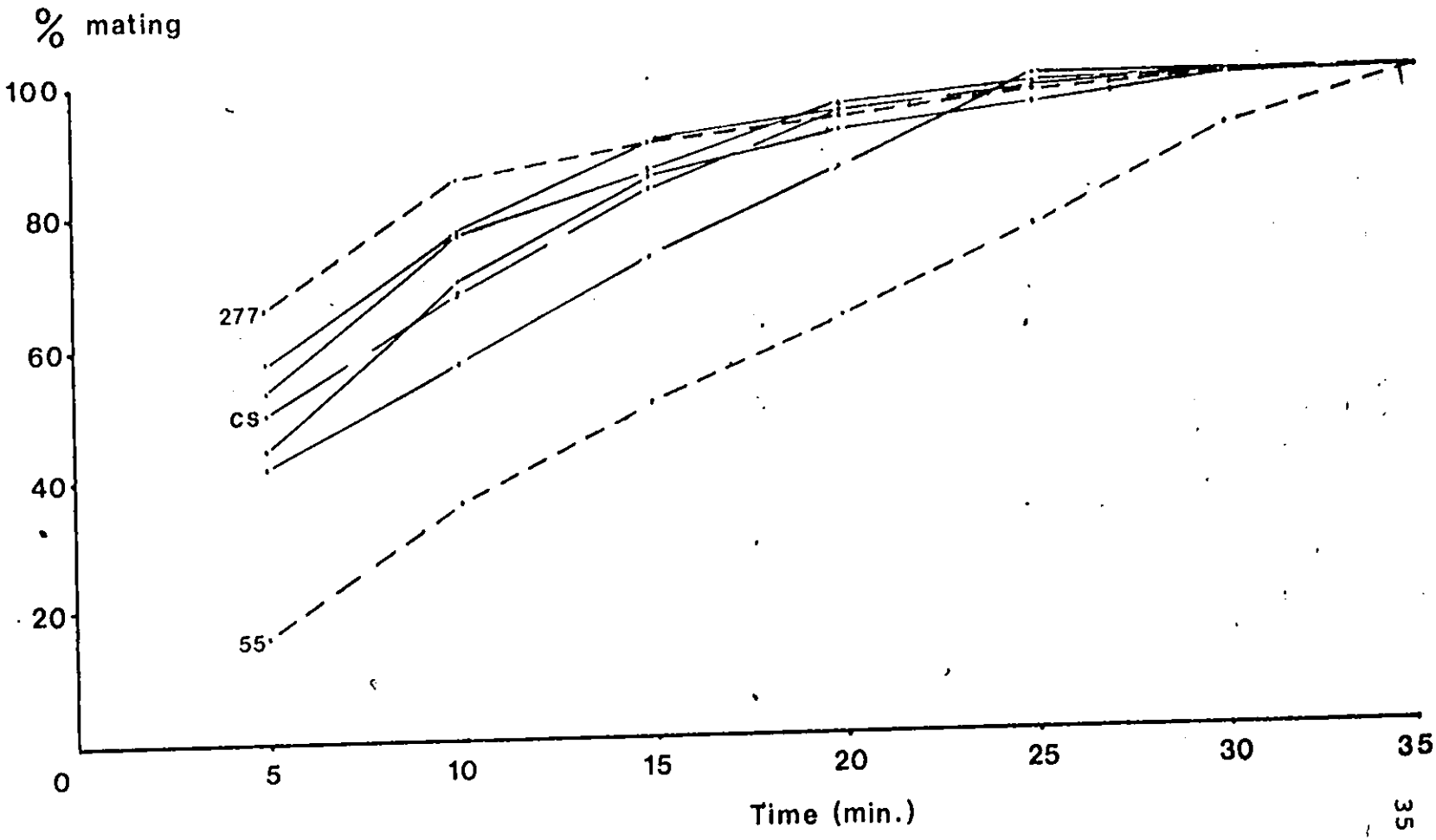


Figure 4b. Cumulative Distributions: Initial Data (mated pairs)



in tabular form in Figure 3 and displayed as cumulative distribution functions (CDF's) in Figure 4, where two graphing methods have been used. Figure 4a shows the percentage of pairs set that began copulation at a given time, while Figure 4b displays the percentage of total copulations observed versus time. Figure 4, and particularly 4a, illustrates some important points: (1) The CS reference curve indicates that after 35 minutes, approximately 70-80% of males mated under the conditions of the test. (2) The four derived strains (nos. 45, 250, 271 and 274) in solid line were not significantly different from CS (see Appendix 3, groups 1 to 3 for tabulated values of the Kolmogorov - Smirnov statistic). Together these curves represent a "main sequence" of mating distributions from which the others deviated to a greater or lesser degree. (3) Three derived strains demonstrated significant departures from the main sequence: Line 263 showed a marked reduction in the frequency of copulations in 35 minutes; only 2 of 144 (1.4%) mated. Its lack of mating activity under test conditions contrasted sharply with its apparently normal growth in culture. Line 55 also displayed a reduction in mating activity relative to the main sequence but to a lesser extent. Line 277 exhibited a significantly higher percentage of copulations in comparison to all strains tested. This was

surprising since a more successful strain was not expected to have been produced with the experimental procedure used.

The latter three strains were studied further in characterization experiments, the results of which are described below. Organizationally, they are grouped according to strain with common tests reserved for the final section.

2. Mutant Characterization

a) Line #55

Soon after beginning tests on this line it was discovered to be associated with a morphological mutant wing phenotype. The mutant exhibited variable expression, some flies possessing wings with severe warping and blisters while others appeared as wild type with planar wings. In between these two extremes were those with less severe defects (some warping with no blisters, or small blisters with no warping), as well as those with an asymmetric morphology (one wing affected to a variable extent, the other wild type). Generally, individuals could be classified according to wing morphology into one of four categories: unaffected (or pseudo-wild type), left wing affected, right wing affected, and both wings affected. In all cases, venation was normal, although in severely affected individuals venation could not be assessed.

The presence of this morphological change allowed the elimination of $\overline{XX}$ chromosome from this line by outcrossing to CS (55 ♂ x CS ♀), and then back to strain #55 (55/CS ♀ x 55 ♂). Female progeny from the last cross that showed the mutant phenotype were mated with #55 males, and thereafter this line was cultured inter se.

In order to ascertain whether the observed phenotypic variation was due to underlying genetic variation, different phenotypes were mated to $\overline{XX}$ virgins in single pair crosses. As shown in Figure 5, all phenotypic classes were represented in the progeny of each cross. Moreover, the proportion of flies in each class was roughly constant. As a result, the phenotypic variation of the progeny appeared to be independent of the phenotypic variation between the parents of the different matings.

It was felt that environmental factors might influence the observed proportions. Since temperature is known to affect both larval and pupal development and the expression of some mutant phenotypes, it seemed to be a logical choice as a relevant environmental influence. Accordingly, replicated mass cultures were incubated at 21, 23 25 and 29° Celsius, and progeny were scored for their phenotype.

Figure 6a shows the percentage of affected and unaffected males for each temperature. There is an evident correlation

Figure 5. Progeny Phenotypes from Single Pair Matings

Male Parent Phenotype	Male Progeny Phenotype L	R	B	N
Both wings affected (B)	7	6	10	9
Left wing affected (L)	3	2	2	11
Right wing affected (R)	9	9	6	10
Pseudo-wild type (N)	4	6	2	7

note: progeny are from the mating: strain 55 ♂ x $\overline{XX}$ ♀ ;
all female progeny were unaffected (wild type).

Figure 6a. Strain 55 Phenotypes at Different Temperatures

Temp. (C)	Total Scored	% Mutant Phenotype	Phenotype Class N	L	R	B
21	530	40	319	87	62	62
23	371	54	169	62	82	58
25	382	71	111	87	84	100
29	446	80	91	67	83	205

Figure 6b. Strain 55 Phenotypes at Different Temperatures (°C)

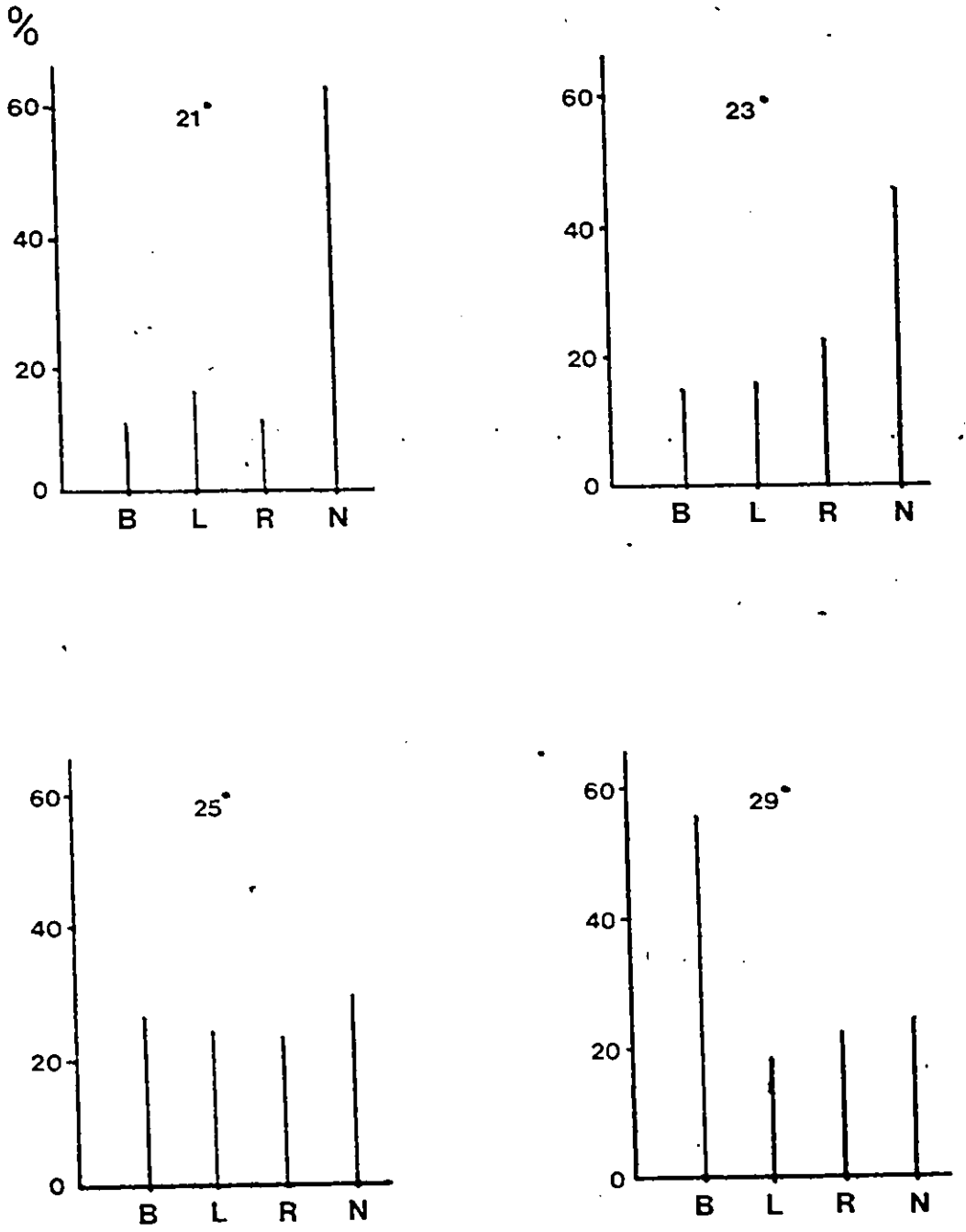


Figure 6c. Statistical Analysis of Figure 6a

i) Preliminary

Group	Count	Mean*	St. Dev.**	St. Error	95% CI for Mean
1	530	.5151	.6962	.0302	.4557 - .5475
2	371	.7008	.7237	.0376	.6269 - .7747
3	382	.9712	.7436	.0380	.8964 - 1.046
4	446	1.2256	.7744	.0367	1.184 - 1.328

* sample calculation:

$$\left[(0\ 319)+(1\ 87)+(1\ 62)+(2\ 62)\right]/530 = .5151$$

** sample calculation:

$$\left\{\left[(0-.5151)^2(319)+(1-.5151)^2(87)+(1-.5151)^2(62)+(2-.5151)^2(62)\right]/529\right\}^{\frac{1}{2}} = .6962$$

ii) Scheffe's Test

- all pairwise comparisons made to yield homogeneous subsets at $\alpha = .05$ level

	Subset 1	Subset 2	Subset 3	Subset 4
Group(s)	1	2	3	4
Mean	.5151	.7008	.9712	1.2556

Fig. 6c (cont'd)

iii) Analysis of Variance

Source	d.f.	S.S.	M.S.	F ratio	F prob
1. Between Groups	3	146.6674	48.8891	90.9040	0.0000
linear (unique)	1	146.1978	146.1978	271.839	0.0000
linear (step)	1	145.5359	145.5359	270.608	0.0000
dev. from linear	2	1.1315	0.5657	1.052	0.3497
quadratic (unique)	1	1.0317	1.0317	1.918	0.1663
quadratic (step)	1	1.0352	1.0352	1.925	0.1656
dev. from quad.	1	0.0962	0.0962	0.179	0.6724
2. Within Groups	1725	927.7227			
3. Total	1728	1.74.3901			

between culture temperature and the frequency of affected males. Also tabulated in Figure 6a are the counts for the three classes of affected phenotypes. The graphical presentation of this data (Figure 6b) illustrates the trend towards more severe wing defects with increasing temperature.

Statistical analysis (Figure 6c) was done at the McMaster Computing Facility using the systems library program SPSS (Statistical Package for the Social Sciences). To permit this analysis the phenotypic distribution was represented numerically by using weightings of 0, 1, and 2 for classes N, L and R, and B respectively. Weightings thus reflected the severity of the phenotype regardless of the symmetry of effect. Using this scheme the average (numerical) phenotype and standard deviation could be calculated for each temperature (Figure 6c,i). The 95% confidence intervals for the four means did not overlap, indicating that temperature has a significant influence on phenotypic distributions. The more rigorous Scheffe's test was aimed at constructing homogenous subsets of the four groups at a 95% confidence level (Figure 6c,ii). All groups were seen to be unique, and the homogenous subsets were composed of only one member each. The analysis of variance (Figure 6c,iii) confirmed that there was a highly significant added variance component among temperature groups for fly phenotype. Results

of tests for trends indicated that the average phenotype varied with temperature in a linear fashion, the quadratic components not contributing significantly to between groups variation.

This analysis taken as a whole shows that the expression of mutant 55 is conditioned by the temperature at which flies were raised. Because all phenotypic classes were represented at each temperature, this mutant was deemed to be temperature-influenced rather than temperature-sensitive.

As a strain with a morphological marker, flies could be scored without extensive behavioural testing, and strain 55 was amenable to standard genetic mapping techniques. It was suspected that the mutation was sex-linked because only males were observed with the mutant phenotype while the line was carried with $\overline{XX}$ females. Consequently, mapping crosses were performed using a multiply-marked X-chromosome stock (as described in Methods, part 1), and these are shown in Figure 7a. F2 male progeny were scored for phenotype. The fact that some flies of this strain appear as wild type necessitated test crossing some F2 males to $\overline{XX}$ virgins and scoring males in the next (F3) generation. The results of the mapping and test crosses are given in Figure 7b, and data analysis in Figure 7c. The data indicate that mutant 55 is sex-linked and lies between y and cv.

Figure 7a. Mapping Crosses for Strain 55

1. P 55/55 ♀ x y cv v f ♂

   ↓

   F1 55/y cv v f ♀

2. F1 55/y cv v f ♀ x y cv v f ♂

   ↓

   F2 -recombinant progeny; males scored for phenotype

Figure 7b. Progeny Phenotypes from Mapping Crosses

Phenotype	No.	Phenotype	No.
$y^+ cv^+ v^+ f^+$ 55 (parental)	260	+ + + + wild	7
+ + + − "	82	+ + + − "	8
+ + − + "	15	+ + − + "	4
+ − + + "	4	+ − + + "	8
− + + + "	73	− + + + "	3
+ + − − "	82	+ + − − "	9
+ − + − "	1	+ − + − "	0
+ − − + "	2	+ − − + "	17
− + − + "	0	− + − + "	0
− + + − "	22	− + + − "	4
− − + + "	17	− − + + "	68
+ − − − "	3	+ − − − "	52
− + − − "	7	− + − − "	9
− − + − "	3	− − + − "	18
− − − + "	2	− − − + "	87
− − − − "	2	− − − − " (parental)	173

Figure 7c. Data Analysis of Mapping Results

1. Total progeny scored: 1042

2. Marker distances:

markers	recombinant phenotype		count	map distance
y - cv	y	+	118	$\frac{205}{1042}$ = 19.7%
	+	cv	87	
cv - v	cv	+	119	$\frac{245}{1042}$ = 23.5%
	+	v	126	
v - f	v	+	127	$\frac{265}{1042}$ = 25.4%
	+	f	138	

3. 55-- marker distances:

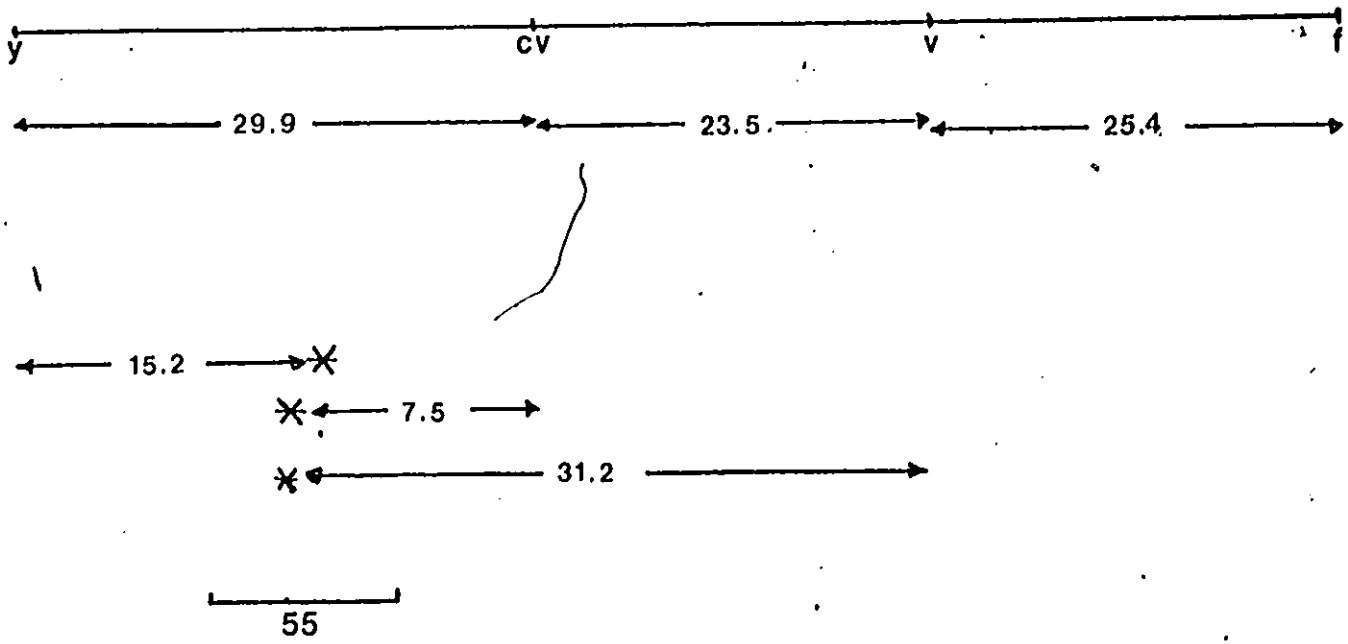
markers	recombinant phenotypes		count	map distance
y - 55	y	55	53	$\frac{158}{1042}$ = 15.2%
	+	+	105	
cv - 55	cv	55	34	$\frac{78}{1042}$ = 7.5%
	+	+	44	
v - 55	v	55	113	$\frac{229}{1042}$ = 22.0%
	+	+	116	

4. Double crossover events (assume gene order: y,55,cv,v,f)

markers	recombinant phenotypes			count	map distance
y - cv	y	55	cv	24	$\frac{52}{1042}$ = 5.0%
	+	+	+	28	
					19.7 + 10.0 = 29.7%
55 - v	55	cv	+	25	$\frac{47}{1042}$ = 4.5%
	+	+	v	22	
					22.0 + 9.0 = 31.0%

Figure 7c. Data Analysis

5. Genetic map



Mating Tests

Strain 55 was originally isolated and characterized as a line with reduced mating success. Once its morphological effect was identified, the question presented itself: Given the altered wing phenotype and the importance of wing display in D. melanogaster courtship, is there a relationship between the severity of wing defect and reduction of mating success? To answer this question, sufficient numbers of strain 55 flies were raised to test 100-150 males of each phenotype with $\overline{XX}$ females. Tests were conducted in the same manner as previously described in Methods, section 3, and the results given in Figure 8. The salient features of Figure 8, and particularly Figure 8b, are the following: (1) the mating frequency curves for phenotype classes 55L, 55R, and 55B are not significantly different from each other at any time plotted (see Appendix 3, group 11); (2) these curves are not significantly different from that of CS males (Appendix 3, groups 8 and 11); (3) the 55N male class is significantly different from both CS and the other classes. Specifically, this difference is manifested in the first five minutes of the test when 55N has a higher mating frequency than all others. This initial superiority decreases after 5 minutes, and from 15 minutes to the completion of the test is insignificant. The 55B class tends to retain its

Figure 8a. Mating Activity Data for Strain 55 Phenotype Classes.

i) 55L - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	15	9	1				1	26	33
2	9	4	2	2	3		1	21	23
3	9	2		1				12	18
4	1			2	2	1	1	7	12
5	12	2	1		1		2	18	19
6	16	2	1	1				20	24
7	9	4	1	2	1			17	19
Total	71	23	6	8	7	1	5	121	148

Cumulative frequencies (%):

48.0	63.5	67.6	73.0	77.7	78.4	81.8	(attempts)
58.7	77.7	82.6	89.2	95.0	95.8	100	(copulations)

ii) 55R - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	8	4	7		3		2	24	32
2	15	2	1	1			1	20	21
3	3	2	5		1			11	11
4	10	5	2	1	2		1	21	21
5	13			1	2	1		17	19
6	13	3						16	19
7	17	3				1		21	24
Total	79	19	15	3	8	4	4	130	147

Cumulative frequencies (%):

53.7	66.7	76.9	78.9	84.3	85.7	88.4	(attempts)
60.8	75.4	86.9	89.2	95.4	96.9	100	(copulations)

Fig. 8a (cont'd)

iii) 55B - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	17	6	4			1		28	32
2	9	4	3			1	1	18	24
3	7	4		1				12	16
4	2	2	1					5	10
5	1	2	1	1		2	1	8	13
6	8	2	3	1	1		2	17	19
7	15	2	2	1				20	28
Total	59	22	14	4	1	4	4	108	142

Cumulative frequencies (%):

41.5	57.0	66.9	69.7	70.4	73.2	76.1	(attempts)
54.6	75.0	87.9	91.6	92.5	96.2	100	(copulations)

iv) 55N - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	4	2			1	1		8	10
2	9	4	3					16	20
3	15	2	2			1		20	22
4	16	2	2					20	21
5	38	3	1		1	1		44	48
6	16	2	1					19	19
Total	98	15	9	0	2	3	0	127	140

Cumulative frequencies (%):

70.0	80.7	87.1	87.1	88.6	90.7	90.7	(attempts)
77.7	89.0	96.1	96.1	97.7	100	100	(copulations)

Figure 8b. Cumulative Distributions: Strain 55 Phenotypes

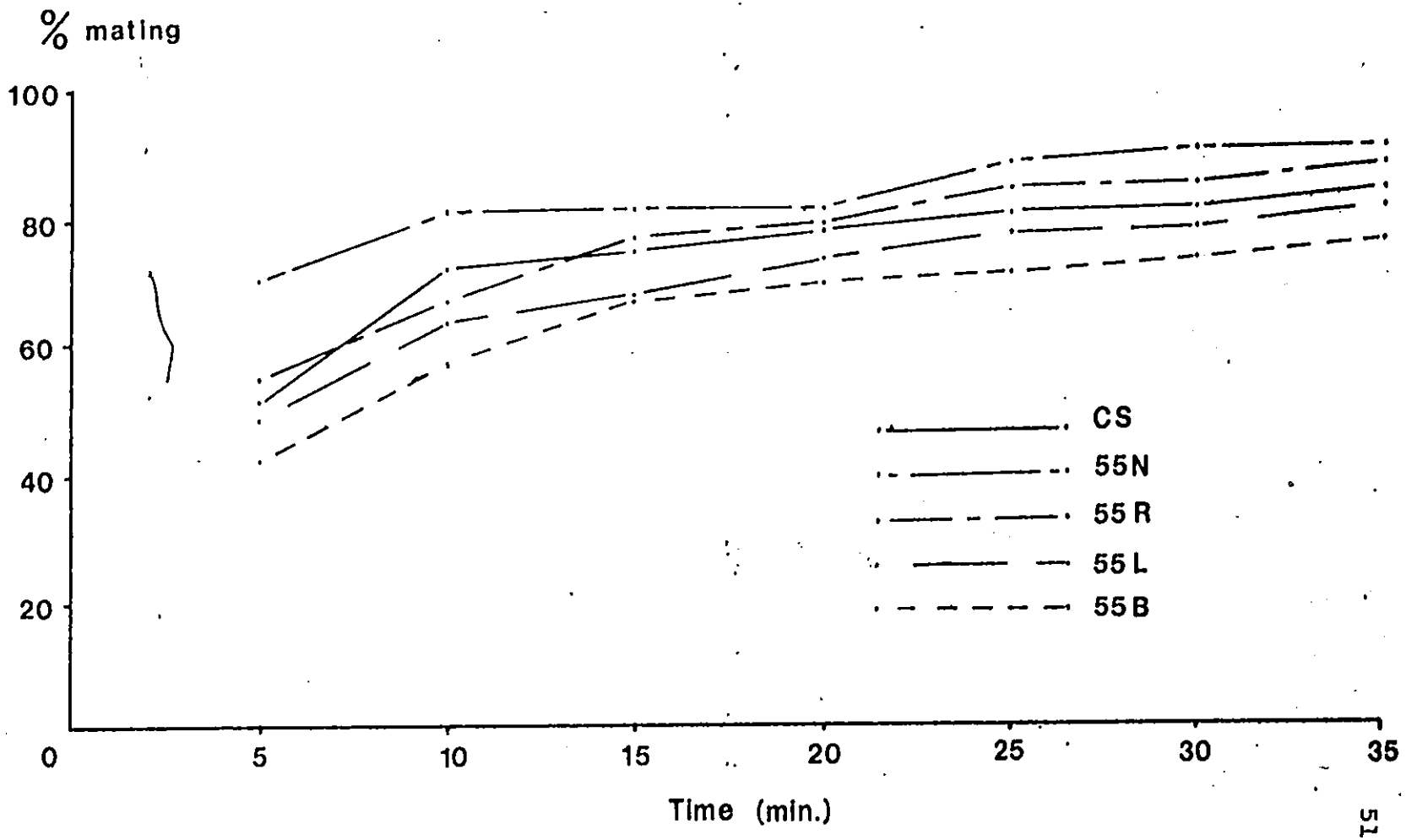
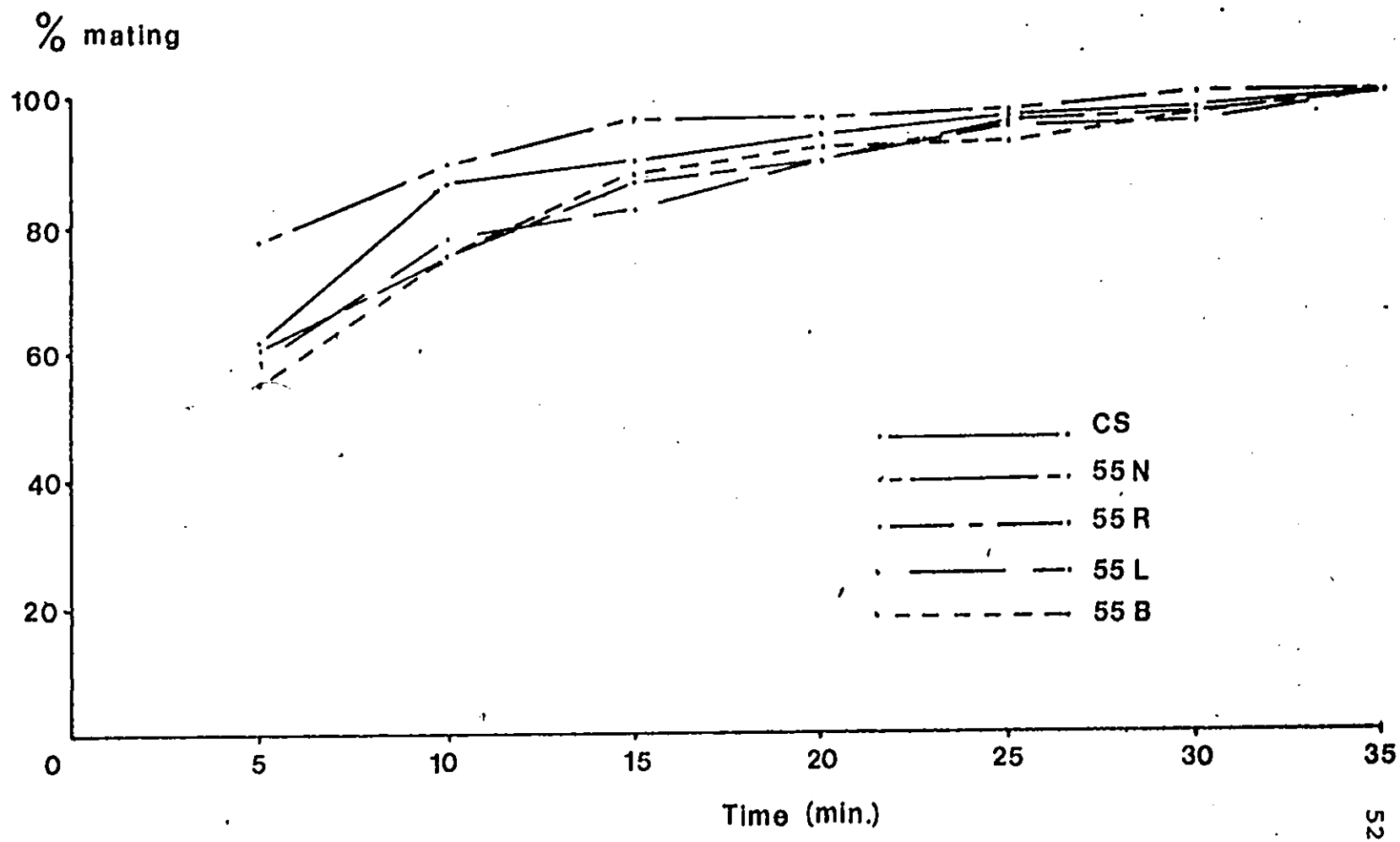


Figure 8c. Cumulative Distributions: Strain 55 Phenotypes (mated pairs)



reduced mating frequency relative to 55N until later times; (4) all classes of strain 55 have shown a marked increase in mating frequency compared to the original 55 data (compare Figure 4a and 8b). Although this may appear to be a problem in reproducibility, other results (for 263 and 277) suggest a more plausible explanation. This phenomenon is treated in more detail in the ensuing discussion.

b) Line #263

Unlike strain 55, 263 exhibited no observed change in external morphology, although it persisted in demonstrating little or no sexual behaviour under test conditions. Observations on the behaviour of 263 males in the presence of virgin females suggested three possible areas responsible for this:

(i) The most obvious difference between 263 and all other lines used in this study was its reduced locomotor activity. Instead of engaging in normal "exploratory" behaviour when placed in the mating tubes (prior to showing courtship activity), strain 263 males tended to remain in a small area of the chamber. Consequently there were fewer meetings between the male and $\overline{XX}$ female, thus reducing the number of opportunities for courtship and copulation.

(ii) On those occasions when male and female met, the

majority of males did not respond with courtship - rather, they remained motionless or turned away from the female. In this sense, they appeared to lack general sexual motivation.

(iii) Even more infrequent than a chance meeting of the sexes were cases where the male initiated normal courtship behaviour. However, despite the fact that all the elements of courtship were present and that the female displayed no obvious rejection responses, intromission was achieved only after several attempts at genital contact by the male. The chief difficulty was apparently an inappropriate or incorrect positioning of the male's curled abdomen.

All of these observations could account for or contribute to the resultant low copulation frequency of strain 263. Further consideration of these points is given in the Discussion section.

Genetic Tests

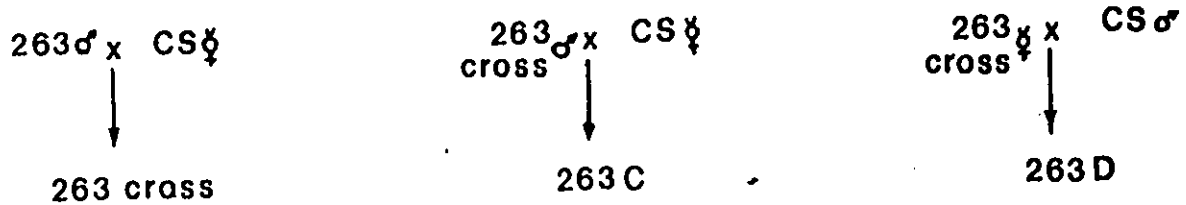
The mutagenesis scheme used to generate all lines in these experiments was directed towards the isolation of sex-linked mutations. The genetics of the system guaranteed that males in a given line would possess genetically identical X-chromosomes while the autosomes were expected to assort randomly in the population. Any CS autosome from the original mutagenized sperm would consequently be present in the

population at a frequency of 25%.

It was deemed necessary to localize the factor responsible for 263's behaviour more positively, however. Besides the obvious need to verify the probable results of the mutagenesis method, two other considerations led to testing the mode of inheritance of 263's behaviour. First, all lines were known to have been subjected to a severe reduction in population as a result of an overheating incubator at generation 3. The testing of populations was done over a period of approximately 8 generations and thus would have been done on lines in which at least one major opportunity for the frequency of an autosomal mutation to increase substantially had occurred. Secondly, in estimating the frequency of CS autosomes in the population, use is made of the implicit assumption that all chromosomal combinations between mutagenized CS males and $\overline{XX}$ females are equally likely and equally fit. In fact this may not be the case.

Regardless of the exact mechanism of its establishment, population homozygosity is the most probable alternative of autosomal models given the fixity of the phenotype in the population and in time. For efficiency, a method was devised that would allow discrimination between the three possible methods (sex-linked, autosomal dominant, and autosomal

Figure 9. Strain 263: Derived Stocks



Predicted Ratios

Model	S : F	S : F	S : F
1 autosomal dom.	1 : 0	1 : 1	1 : 1
2 autosomal recess.	0 : 1	0 : 1	0 : 1
3 sex linked	0 : 1	0 : 1	1 : 1
Result	0.09 : 1	0.13 : 1	0.77 : 1

recessive) at once. The mass matings all involved the laboratory CS stock and are diagrammed in Figure 9. Also listed are the (behavioural) phenotypic ratios of progeny expected under each of the three models, where S denotes slow sexual activity (as observed with 263 males) and F signifies fast or wild-type mating activity.

It is important to note that the predicted ratios in Figure 9 were calculated assuming homozygosity in the parent population. This is reasonable for the sex-linked model since the genetics of the system insured that all males must share the same X-chromosome. If this assumption is relaxed in the case of the autosomal models, however, only the ratios for the autosomal dominant model are affected. Nevertheless, even with a gene frequency of less than 100% in the parent population, the relationship between expected behavioural ratios in the reciprocal cross progeny (263C, 263D) is invariant; for 263C and 263D males, phenotypic ratios will always be equal for the autosomal models (both dominant and recessive), but unequal for the sex-linked model.

The results of the mating tests on these progenies are shown in Figure 10 (see also Appendix 3, groups 9 and 12). Discrimination between the various models is achieved by noting the following:

1) 263-cross and 263C exhibit normal to high mating

success and are not significantly different from each other. 263-cross males do not show the paternal behaviour and this conflicts with the autosomal dominant model. Consequently, this result supports both of the alternative models.

2) 263D shows a significantly lower frequency of males mating at all times relative to 263-cross and 263C males. Thus, a large proportion of 263D males do not copulate during the 35-minute test period. About 65% of 263D males mate, compared to approximately 95% for 263C and 263-cross. Because the reciprocal cross progenies (263C and 263D) are significantly different, this data supports the sex-linked model.

3) For reference, the 263 and CS curves are shown in Figure 10b. It is interesting to note that the sex-linked model predicts genotypic (and therefore behaviourally phenotypic) ratios of 1:0 (fast: slow, or wild: mutant) for both 263-cross and 263C, but these males are significantly more successful than wild type CS males. As first observed with strain 55, there is a deviation from expectation in the direction of more successful mating activity. Further comments on this are made in the Discussion.

An additional prediction of the sex-linked model is that not only should the population of 263D males mate at about half the frequency of wild type, but in addition those males

Figure 10a. Mating Activity Data for Strain 263 Derived Populations.

i) 263 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1								0	12
2								0	10
3			1					1	10
4								0	12
5		1						1	12
6								0	25
7								0	19
8								0	44
Total	0	1	1	0	0	0	0	2	144

Cumulative frequencies (%):

0	0.7	1.4	1.4	1.4	1.4	1.4	(attempts)
0	50	100	100	100	100	100	(copulations)

ii) 263 cross - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	31	9						40	40
2	6	8	1					15	15
3	6	3	1					10	10
4	7	2	1		1			11	12
5	22	2						24	24
6	11	8		1			1	21	24
Total	83	32	3	1	1	0	1	121	125

Cumulative frequencies (%):

66.4	92.0	94.4	95.2	96.0	96.0	96.8	(attempts)
68.6	95.0	97.5	98.3	99.1	99.1	100	(copulations)

Fig. 10a (cont'd)

iii) 263C - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	12							12	13
2	17	1	1	1				20	20
3	24	4	1	2		1		32	35
4	27	5						32	34
Total	80	10	2	3	0	1	0	96	102

Cumulative frequencies (%):

78.4	88.2	91.2	93.1	93.1	94.1	94.1	(attempts)
83.3	93.7	95.8	98.9	98.9	100	100	(copulations)

iv) 263D - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	7	3	1	1				12	15
2	14	2	1	2	3			23	39
3	19	4						23	24
4	13	1	1		1			16	33
Total	53	10	3	3	4	0	0	73	111

Cumulative frequencies (%):

47.7	56.8	59.4	62.2	65.8	65.8	65.8	(attempts)
72.6	89.3	90.4	94.5	100	100	100	(copulations)

Figure 10b. Cumulative Distributions: Strain 263 Derived Stocks

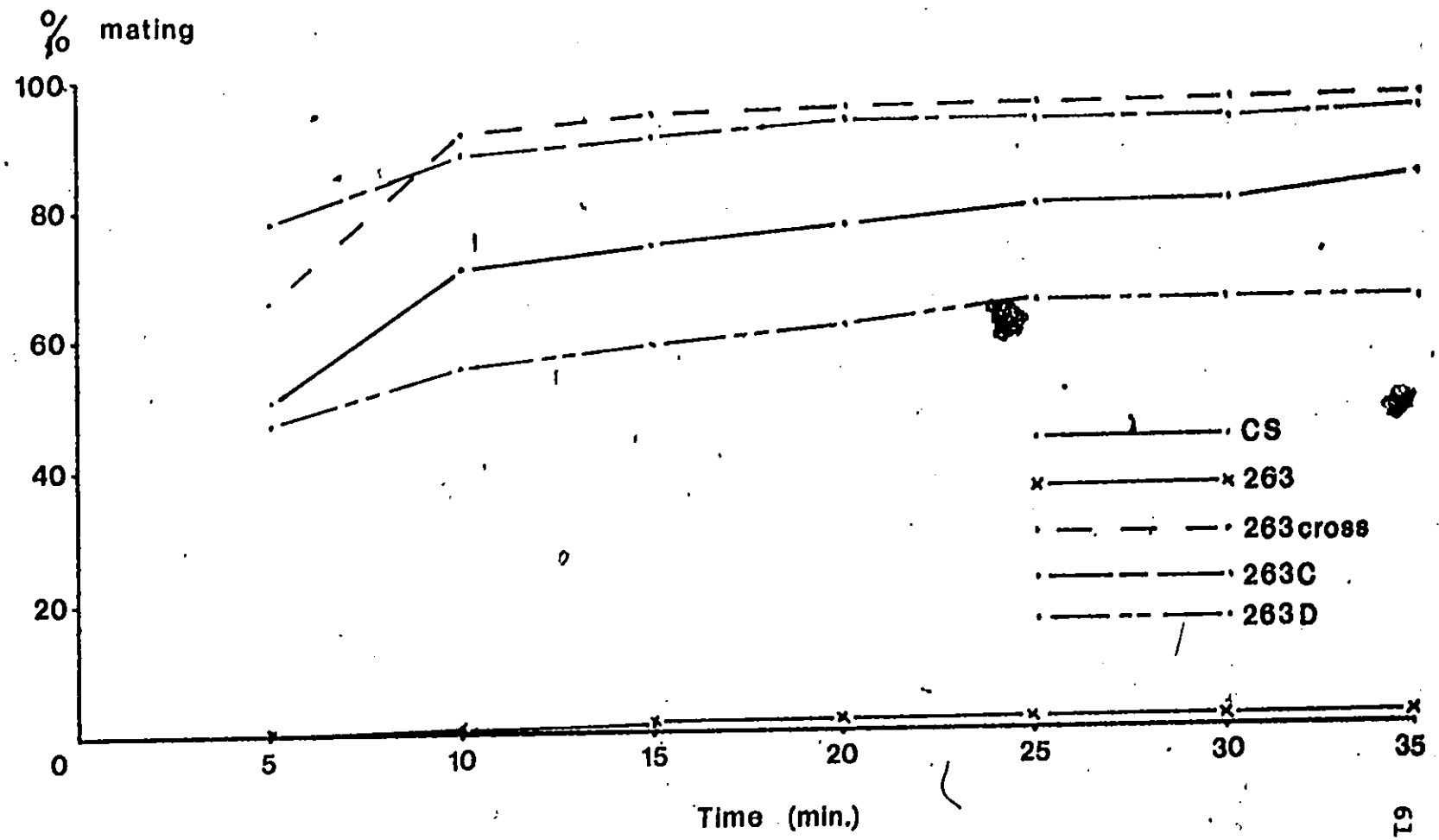
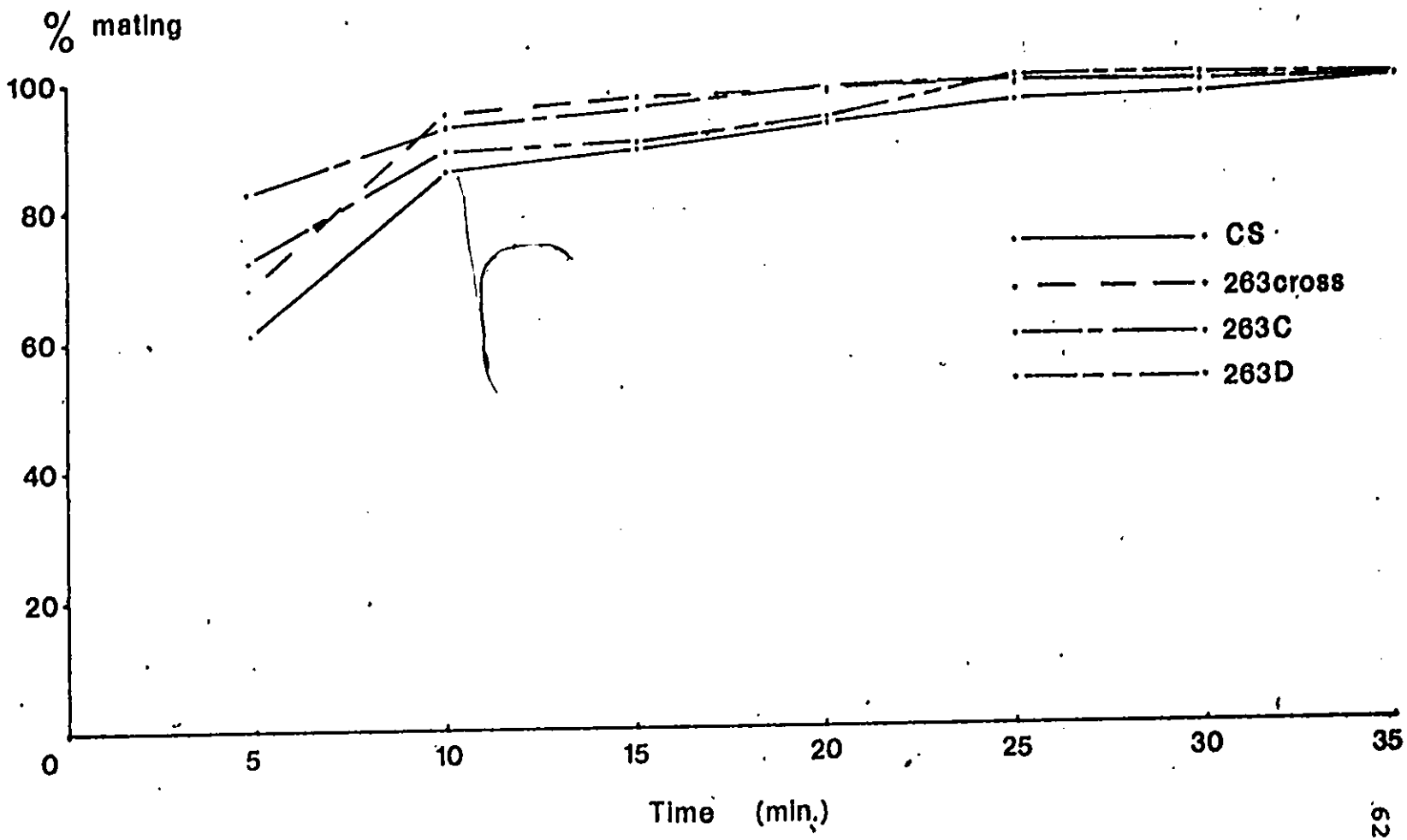


Figure 10c. Cumulative Distributions: Strain 263 Derived Stocks (mated pairs)



that mate (or, in other words, those that are genotypically wild type) should do so with the same relative distribution of copulations in time as CS, 263C, and 263-cross. To see whether this was true, data for CS, 263C, 263D, and 263-cross were replotted using percentage of matings occurring and time as the axes. The result (Figure 10c) shows that there are no significant differences between any of the cumulative distributions for 263-derived populations. This supports the hypothesis that the slow mating phenotype exhibited by 263 males is due to a sex-linked factor. Moreover, it suggests that its action is an all-or-none process; the males in the 263D population that do mate, do so with the same distribution in time as the "wild-type" derivations of 263.

Locomotor Tests

Although reduced mating success seemed to have been positively attributed to a sex-linked gene, no formal connection between altered mating activity and the three possible sources of difficulty as outlined above had been made. Of the three possibilities, the one that lent itself most easily to quantitative assessment was the reduction in locomotor activity. The males that were tested in the countercurrent apparatus were derived in a similar way as those in the preceding section. In addition, another cross

was made (263-cross ♀ x 263 ♂ → 263/263-cross) as a check on the experimental system. All predictions for the various models are the same as for mating behaviour, except for the additional cross progeny which would give 1:0, 1:1, and 1:1 ratios (slow:fast) for the autosomal dominant, recessive and sex-linked models respectively. Behaviourally, the gross parameters of interest were the number and location of modes in the fractionated population's distribution; 0:1 and 1:0 ratios would give unimodal distributions at opposite poles of the apparatus, and 1:1 distributions would be bimodal.

To provide the necessary connection, the relevant question was: is 263's reduced locomotor activity inherited in the same manner as its reduced mating success? If the answer was negative then 263 would appear to have more than one mutant characteristic, one of which played little or no part in its altered mating success.

The locomotor activity test results are given in Figure 11a. By inspection of Figures 11b and 11c, all populations demonstrated near wild-type behaviour in this test, except for two groups. CS, 263C, 263-cross and 263D had unimodal fast distributions, while 263 males were slow and unimodal and 263/263-cross males were bimodal. A G-test of homogeneity (Figure 11d) confirmed these impressions, with 263C, 263D

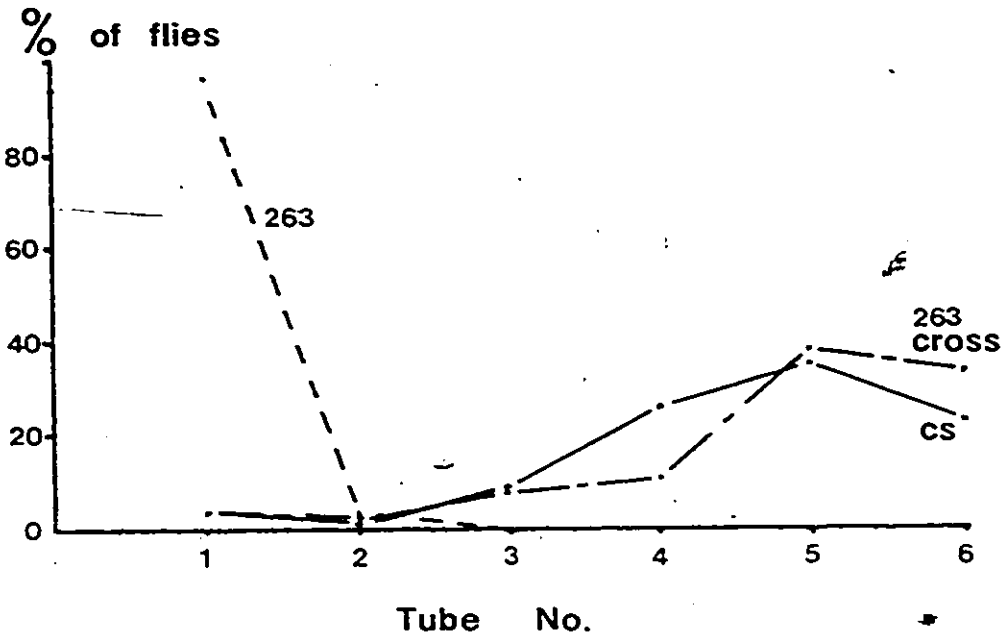
Figure 11a. Locomotor Activity Data from Strain 263 Derived Populations.

		Tube No.						
Strain	Run	1	2	3	4	5	6	Total
CS	1	3	3	9	23	26	7	71
	2	3	0	5	14	24	25	71
263	1	79	2	0	0	0	0	81
	2	78	3	0	0	0	0	81
263 cross	1	4	1	4	5	24	25	63
	2	1	3	6	10	24	19	63
263C	1	1	0	6	6	19	31	63
	2	0	1	3	4	24	30	62
263D	1	0	4	2	7	33	29	75
	2	2	2	2	8	11	27	52
263/	1	27	3	1	3	13	13	60
263 cross	2	28	0	3	1	9	19	60

increasing activity

Figures 11 b, c. Locomotor Activities:

263 Derived Strains



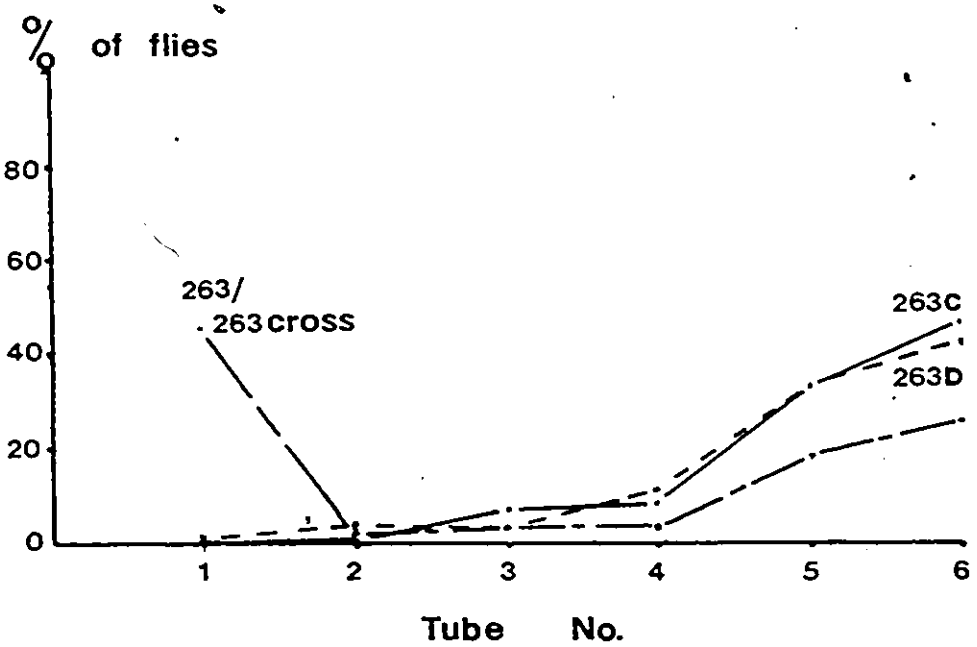


Figure 11d. Statistical Analysis of Figure 11a.*

i) Table 1.

Strain (a=6)	Run (b=2)	Tube No. (c=6) 1	2	3	4	5	6	Total	Avg.
CS	1	3	3	9	23	26	7	71	
	2	3	0	5	14	24	25	71	
	Total	6	3	14	37	50	32	142	4.55
263	1	79	2	0	0	0	0	81	
	2	78	3	0	0	0	0	81	
	Total	157	5	0	0	0	0	162	1.03
263 cross	1	4	1	4	5	24	25	63	
	2	1	3	6	10	24	19	63	
	Total	5	4	10	15	48	44	126	4.82
263C	1	1	0	6	6	19	31	63	
	2	0	1	3	4	24	30	62	
	Total	1	1	9	10	43	61	125	5.21
263D	1	0	4	2	7	33	29	75	
	2	2	2	2	8	11	27	52	
	Total	2	6	4	15	44	56	127	5.06
263/263 cross	1	27	3	1	3	13	13	60	
	2	28	0	3	1	9	19	60	
	Total	55	3	4	4	22	32	120	3.26
TOTAL		226	22	41	81	207	225	802	

*based on analysis of Sokal and Rohlf (1969), pp. 582-584, 602-606.

Figure 11d. (cont'd)

Table 2.

	Tube No.						
Run	1	2	3	4	5	6	Total
1	114	13	22	44	115	101	413
2	112	9	19	37	92	120	389
Total	226	22	41	81	207	225	802

ii) Preliminary calculations.

A (cells) : $\Sigma f \ln f = 3\ln 3 + 3\ln 3 + 9\ln 9 + \ldots + 19\ln 19 = 2512.096$

B (a x b) : $71\ln 71 + 71\ln 71 + 81\ln 81 + \ldots + 60\ln 60 = 3376.735$

C (a x c) : $6\ln 6 + 3\ln 3 + 14\ln 14 + \ldots + 32\ln 32 = 3044.261$

D (b x c) : $114\ln 114 + 13\ln 13 + 22\ln 22 + \ldots + 120\ln 120 = 3570.410$

E (a) : $142\ln 142 + 162\ln 162 + 126\ln 126 + \ldots + 120\ln 120 = 3930.540$

F (b) : $143\ln 143 + 389\ln 389 = 4807.517$

G (c) : $226\ln 226 + 22\ln 22 + 41\ln 41 + \ldots + 225\ln 225 = 4123.746$

H : $802\ln 802 = 5363.061$

Fig. 11d (cont'd)

iii) Significance tests.

Overall G = 2(A-E-F-G+2H) = 752.83 on abc-a-b-c+2 = 60 d.f. $p \ll .005$

Independence of distribution vs. run:

G = 2(D-F-G-H) = 4.416 on bc-b-c+1 = 5 d.f. n.s.

Independence of distribution vs. strain:

G = 2(C-E-G+H) = 666.072 on ac-a-c+1 = 25 d.f. $p \ll .005$

Independence of run vs. strain:

G = 2(B-E-F+H) = 3.478 on ab-a-b+1 = 5 d.f. n.s.

∴ significant heterogeneity overall is due to variation between strains.

Fig. 11d (cont'd)

iv.) Partitioning of strain variation: homogeneous groups.
- comparisons made between calculated and critical G value (critical G at α=.05 level on 25 d.f., G_{crit}=37.7)

largest homogeneous groups:

1) CS - 263 cross - 263C

$G = 2[61\ln 6+31\ln 3+\ldots+611\ln 611 - (1421\ln 1421+1261\ln 1261+\ldots +1321\ln 1321)+3931\ln 3931]$

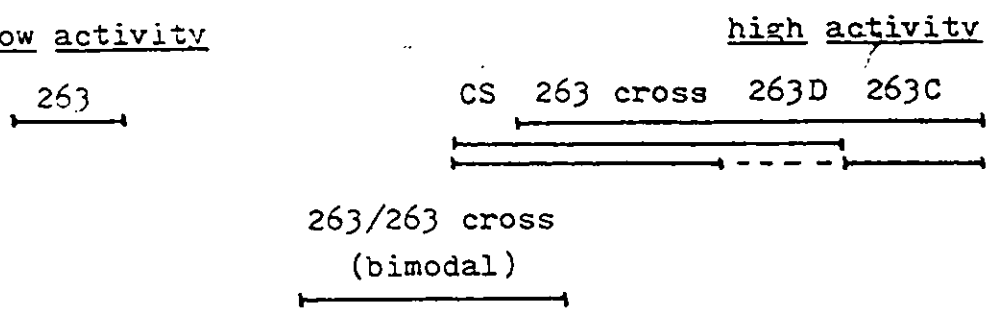
$= 34.99$ n.s.

2) CS - 163 cross - 263D

$G = 2[61\ln 61+31\ln 31+\ldots+561\ln 561 - (1421\ln 1421+1261\ln 1261+\ldots +1321\ln 1321)+3951\ln 3951]$

$= 33.56$ n.s.

other comparisons of calculated and critical G may be made to confirm the following arrangement of homogeneous groups:*



*one horizontal line underscores the menbers of a particular group; note that CS and 263 cross are homogeneous with 263D or 263C, but not both simultaneously

and 263-cross comprising one homogenous set, and the other two 263 populations (263 and 263/263-cross) each behaving in unique ways. CS males were homogenous with 263-cross and either (but not both) 263C or 263D. These results were in agreement with the autosomal recessive model.

This unexpected finding, contrary to those of the mating tests, was retested by crossing 263 males to XX virgins of the original stock (Dr. S.F.H. Threlkeld, private communication) and observing the male progeny. Their slow behaviour served to disprove the initial conclusions of the locomotor tests and confirm the sex-linked model. Consequently, it appeared that both reduced mating success and reduced locomotor activity were inherited in the same manner (i.e., as sex-linked traits) and perhaps were due to a common, single gene mutation.

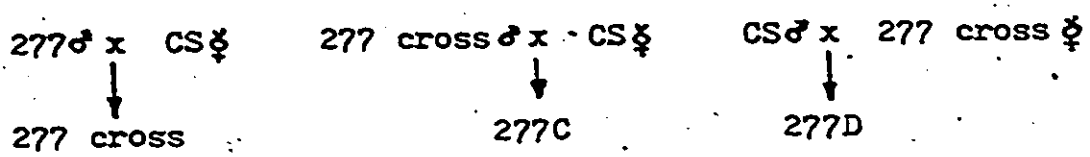
c) Line #277

Like 263, strain 277 was morphologically wild-type. As mentioned previously, however, males showed a significantly higher mating success than all other types tested. For the same reasons outlined for strain 263, and also because its consistently more successful mating behaviour could have constituted a selectively advantageous trait, similar crosses between 277 and CS were done as for 263 to determine the mode of inheritance of its behaviour (Figure 12).

Ideally, the mating behaviour of each male population would give information about this. In contrast to 263 male behaviour which was qualitatively different from normal males, though, 277 males demonstrated a quantitative change from wild-type and this made precise calculation of model predictions difficult. Two male populations had to differ by at least 17% at one or more points in their CDF's for the difference to be identified as statistically significant (Appendix 3). Since the parent CS and 277 males showed a maximum difference of 20% at one point, there was little possibility of detecting more than two distinct groups among the derived male populations.

The models could provide for the construction of expected homogenous groups and this was done as follows (see also Figure 12 for a brief explanation): If a model predicted that males in two populations were genetically identical with respect to the relevant chromosomes (either sex chromosomes or autosomes), then the two populations were placed in the same homogenous group. If there was total dissimilarity between them, then they were "absolutely" unequal and placed in different groups. Intermediate classifications arose from those cases where the corresponding model predicted that two types of males (and behaviour) were present in the population. The anticipated groupings of males were as shown in Figure 12.

Figure 12. Strain 277: Derived Stocks.



Expected Homogeneous Groups*

model	groups
Autosomal dominant	277 = 277 cross ≥ 277C = 277D ≥ CS
Autosomal recessive	277 > 277 cross = 277C = 277D = CS
Sex - linked	277 ≥ 277D ≥ 277C = 277 cross = CS
Observed result	277 ≥ 277 cross ≥ 277C ≥ 277D ≥ CS

*derived populations are listed approximately in decreasing mating frequency and homogeneous groups underlined. The approximation occurs because, unlike strain 263, the parent 277 population does not show a marked difference in behaviour. Consequently, ratios of predicted types (eg. 1:1, mutant: wild) may not be observed as significantly different from either 277 or CS. Here, equality and inequality (=,) denote expected absolute relationships in behaviour, whereas inequalities ( ) reflect 1:1 progeny ratios which may be homogeneous with (dotted lines) 277 or CS.

The results of these tests are given in Figure 13 and may be summarized as follows:

1) Homogenous sets of curves may be distinguished, with one group composed of 277, 277-cross and 277C males, and with CS males in another.

2) The curve for 277D is not significantly different from either of these groups (see Appendix 3, groups 10 and 17). This pattern of differences does not strictly fit any of the models under test since (a) no differences between 277 and its derived populations exist, contrary to the sex-linked and autosomal recessive models; and (b) the autosomal dominant model predicted that both 277C and 277D should bear the same relationship to CS, yet 277C is significantly different from CS while 277D is not. The possible reasons for the failure to discriminate among models are given in the Discussion.

A behavioural explanation for 277's increased mating success was not evident despite the observation of over one hundred test pairs. As a factor in mating behaviour, locomotor activity has been implicated in a variety of ways (see Burnett and Connolly (1974), for a review). In an attempt to ascertain whether this aspect of behaviour was altered, 277 and its derived stocks were subjected to countercurrent experiments. The results of these are given in Figure 14 and

Figure 13a. Mating Activity Data for Strain 277 Derived Populations.

i) 277 - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	23	5	1	1	1		1	32	34
2	11	4				1	1	17	17
3	9	1						10	10
4	7	1	1	1	1			11	11
5	9	1			1			11	12
6	14	2						16	16
7	11	4	2	2				19	19
Total	84	18	4	4	3	1	2	116	119

Cumulative frequencies (%):

70.6	85.7	89.1	92.4	95.0	95.8	97.5	(attempts)
72.4	87.9	91.3	94.7	97.3	98.2	100	(copulations)

ii) 277 cross - $\overline{XX}$

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	18	3	1					22	24
2	18	2	1					21	23
3	9	1						10	10
4	23	2	1					26	26
5	19	1	1	1				22	23
6	22	1	1					24	24
Total	109	10	5	1	0	0	0	125	130

Cumulative frequencies (%):

83.8	91.5	95.4	96.2	96.2	96.2	96.2	(attempts)
87.2	95.2	99.2	100	100	100	100	(copulations)

Fig. 13a (cont'd)

iii) 277C - $\overline{XX}$

Run	Time (min.) 5	10	15	20	25	30	35	Total mated	tested
1	7	2	1					10	11
2	12	3		1				16	16
3	18	3	2	1			1	25	27
4	34	1	1					36	37
Total	71	9	4	2	0	0	1	87	91

Cumulative frequencies (%):

78.0	87.9	92.3	94.5	94.5	94.5	95.6	(attempts)
81.6	91.9	96.5	98.8	98.8	98.8	100	(copulations)

iv) 277D - $\overline{XX}$

Run	Time (min.) 5	10	15	20	25	30	35	Total mated	tested
1	17	3	2		1	1		24	24
2	18	2	3	1	1			25	25
3	20	2	4		1	1		28	33
4	12	5			1			18	19
Total	67	12	9	1	4	2	0	95	101

Cumulative frequencies (%):

66.3	78.2	87.1	88.1	92.1	94.1	94.1	(attempts)
70.5	83.1	92.6	93.7	97.9	100	100	(copulations)

Figure 13b. Cumulative Distributions: Strain 277 Derived Stocks

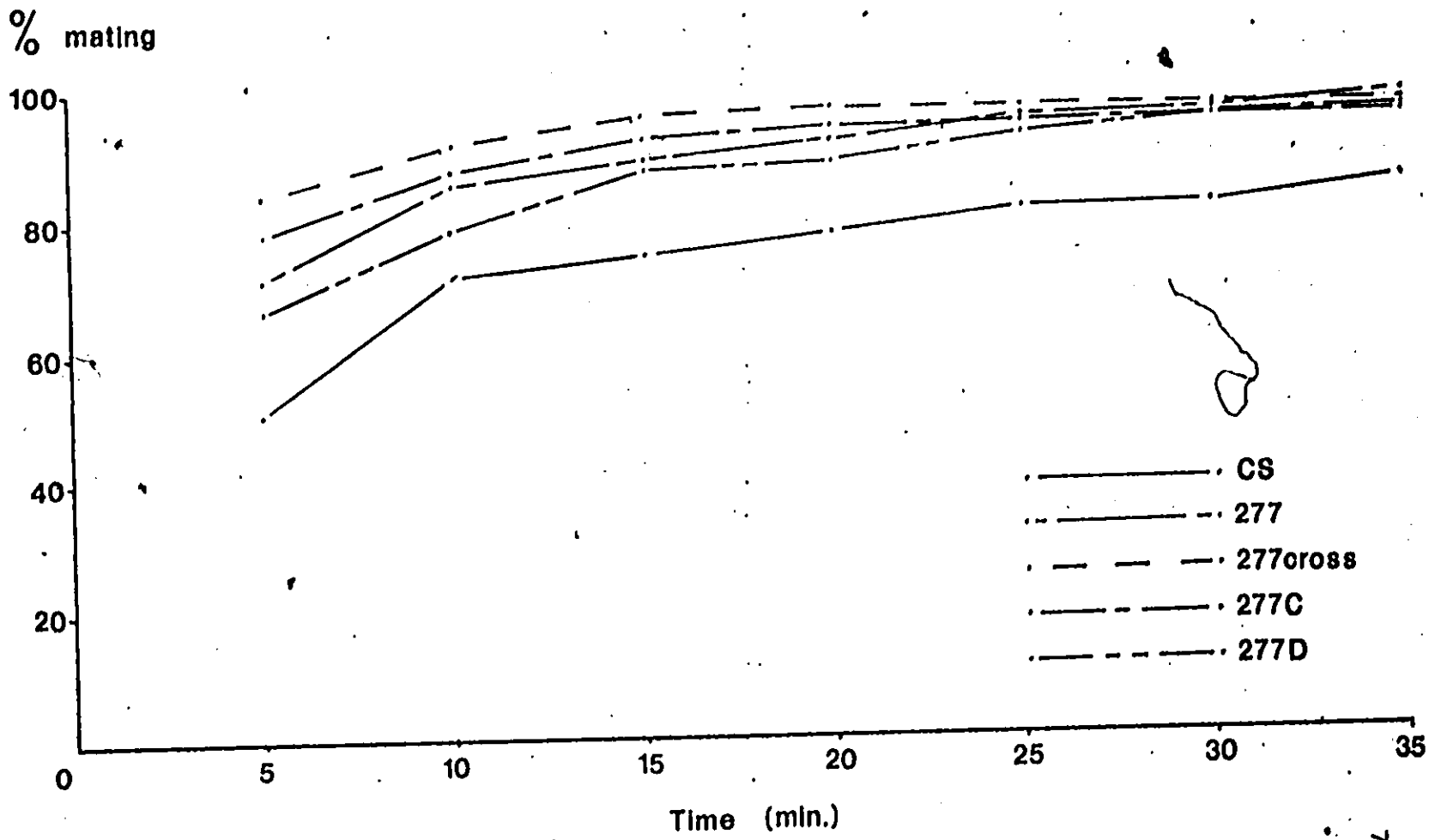


Figure 13c. Cumulative Distributions: Strain 277 Derived Stocks

(mated pairs)

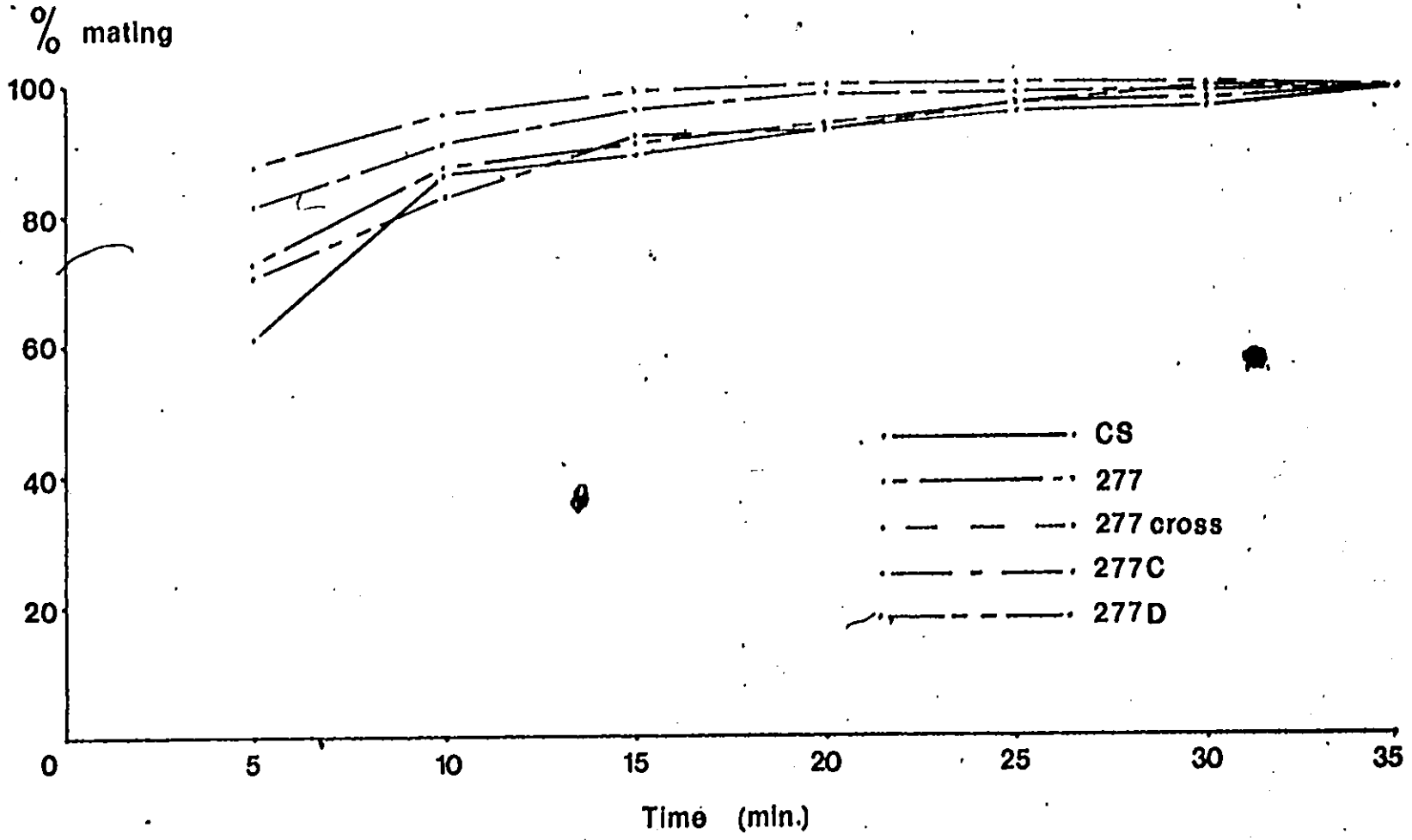


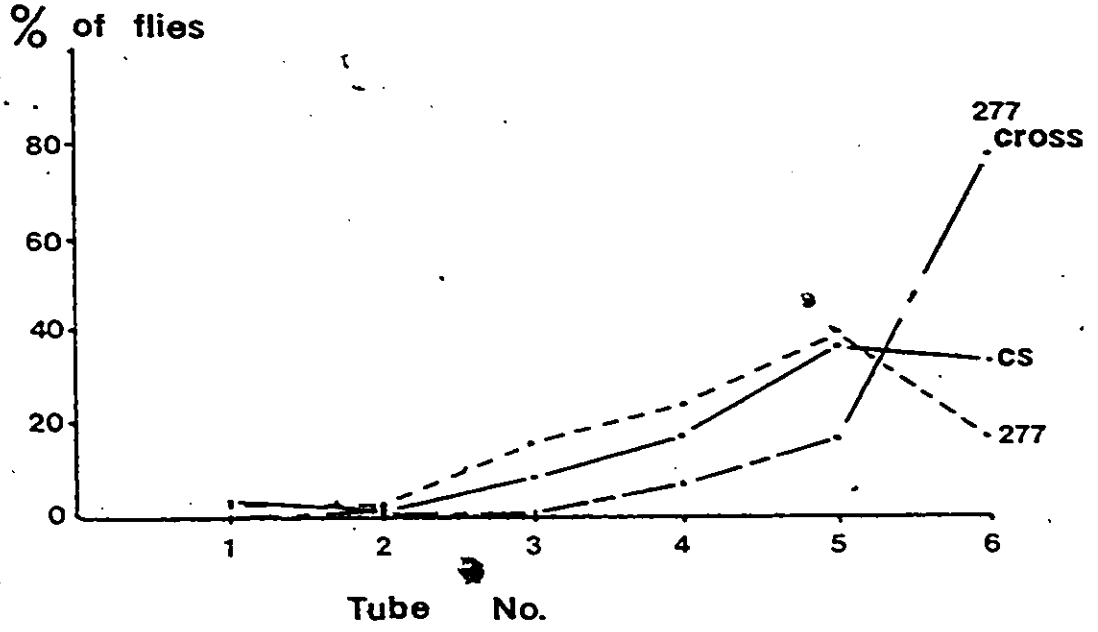
Figure 14a. Locomotor Activity Data from Strain 277 Derived Populations.

Strain	Run	Tube No. 1	2	3	4	5	6	Total
CS	1	4	3	8	13	25	14	77
	2	1	1	5	14	19	35	75
277	1	2	1	11	16	23	13	66
	2	2	3	9	16	28	8	66
277 cross	1	0	0	0	5	7	51	63
	2	0	1	1	3	13	46	66
277C	1	0	2	2	12	10	35	61
	2	0	2	8	15	26	13	64
277D	1	5	4	7	14	11	12	53
	2	5	11	6	8	13	10	53
277/ 277 cross	1	0	2	5	9	17	34	67
	2	1	4	7	14	24	14	64

increasing activity

Figures 14b, c. Locomotor Activities:

277 Derived Strains



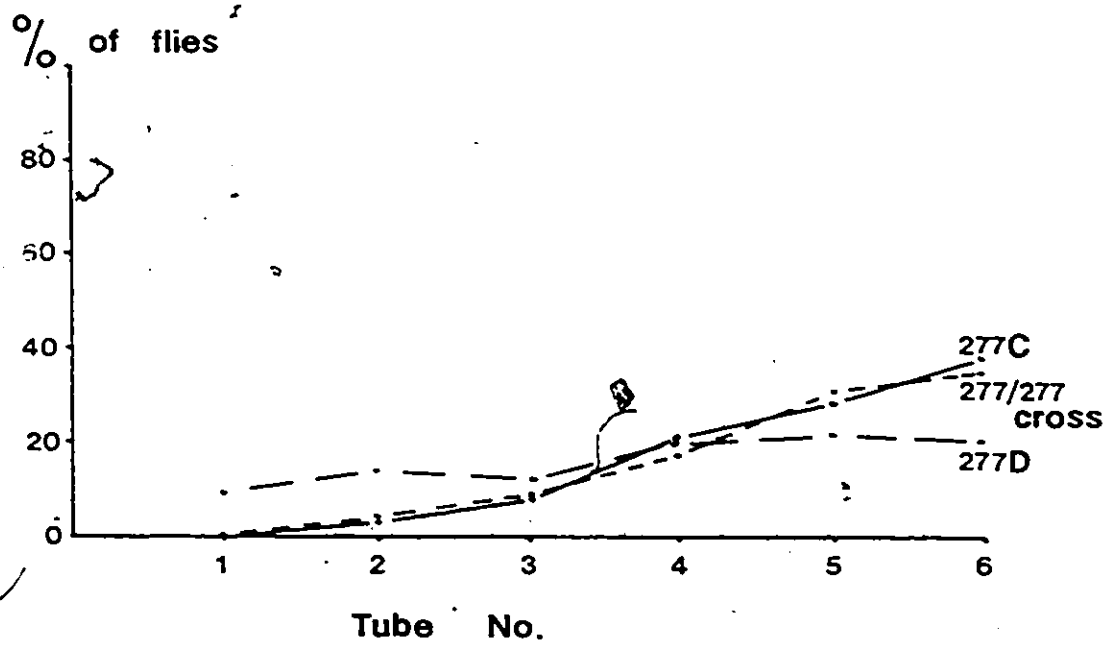


Figure 14d. Statistical Analysis of Figure 14a.*

i) Table 1.

Strain (a=6)	Run (b=2)	Tube No. (c=6) 1	2	3	4	5	6	Total	Avg.
CS	1	4	3	8	13	35	14	77	
	2	1	1	5	14	19	35	75	
	Total	5	4	13	27	54	49	152	4.73
277	1	2	1	11	16	23	13	66	
	2	2	3	9	16	28	8	66	
	Total	4	4	20	32	51	21	132	4.40
277 cross	1	0	1	1	3	13	46	64	
	2	0	0	0	5	7	51	63	
	Total	0	1	1	8	20	97	127	5.66
277C	1	0	2	8	15	26	13	64	
	2	0	2	2	12	10	35	61	
	Total	0	4	10	27	36	48	125	4.91
277D	1	5	4	7	14	11	12	53	
	2	5	11	6	8	13	10	53	
	Total	10	15	13	22	24	22	106	3.95
277/	1	0	2	5	9	17	34	67	
277 cross	2	1	4	7	14	24	14	64	
	Total	1	6	12	23	41	48	131	4.84
TOTAL		20	34	69	139	226	285	773	

*based on analysis of Sokal and Rohlf (1969), pp. 582-584, 602-606.

Fig. 14d (cont'd)

Table 2.

	Tube No.						
Run	1	2	3	4	5	6	Total
1	11	13	40	70	125	132	391
2	9	21	29	69	101	153	382
Total	20	34	69	139	226	285	773

ii) Preliminary calculations.

A (cells) : $\Sigma f \ln f = 4\ln 4 + 3\ln 3 + 8\ln 8 + \ldots + 14\ln 14 = 2198.759$

B (a x b) : $77\ln 77 + 75\ln 75 + 66\ln 66 + \ldots + 64\ln 64 = 3224.171$

C (a x c) : $5\ln 5 + 4\ln 4 + 13\ln 13 + \ldots 48\ln 48 = 2701.107$

D (b x c) : $11\ln 11 + 13\ln 13 + 40\ln 40 + \ldots + 153\ln 153 = 3462.039$

E (a) : $152\ln 152 + 132\ln 132 + 127\ln 127 + \ldots + 131\ln 131 = 3759.887$

F (b) : $391\ln 391 + 382\ln 382 = 4604.916$

G (c) : $20\ln 20 + 34\ln 34 + 69\ln 69 + \ldots + 285\ln 285 = 3993.856$

H : $773\ln 773 = 5140.666$

Fig. 14d (cont'd)

iii). Significance tests.

Overall $G = 2(A-E-F-G+2H) = 242.864$ on abc-a-b-c+2 = 60 d.f. $p \ll .005$

Independence of distribution vs. run:

$G = 2(D-F-G+H) = 7.866$ on bc-b-c+1 = 5 d.f. n.s.

Independence of distribution vs. strain:

$G = 2(C-E-G+H) = 176.06$ on ac-a-c+1 = 25 d.f. $p \ll .005$

Independence of run vs. strain:

$G = 2(B-E-F+H) = 0.068$ on ab-a-b+1 = 5 d.f n.s.

∴ significant heterogeneity overall is due to variation between strains.

Fig. 14d (cont'd)

iv) Partitioning of strain variation: homogeneous groups.
- comparisons made between calculated and critical G value (critical G at α=.05 level on 25 d.f., G_{crit}=37.7)

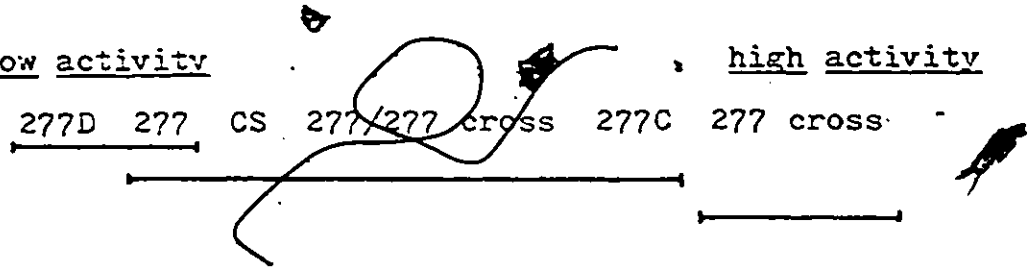
largest homogeneous group:

277C - 277/277 cross - CS - 277

$$G = 2[51\ln 5+41\ln 4+\ldots+481\ln 48 - (1521\ln 152+1321\ln 132+\ldots 1661\ln 166) + 5401\ln 540]$$

$$= 32.208 \qquad \text{n.s.}$$

other comparisons of calculated and critical G may be made to confirm the following arrangement of homogeneous groups:*



*one horizontal line underscores the members of a particular group

present a confusing behavioural picture. CS and 277 males are members of a homogenous set with 277C and 277/277-cross. 277-cross is unique and at one end of this central group, while 277D is homogenous only with 277 at the other extreme. No model accounts for the heterogeneity of 277-derived males, given an initial homogeneity between the parent populations, CS and 277.

3. Comparison Among Lines: Mating in the Absence of Light

The mating tests conducted in the dark were intended to determine how these strains behaved without the visual cues that are known to play a role in the mating activity of D. melanogaster. (Spieth, 1950, 1952; Grossfield, 1970; Hartman, 1963). Although this species does not require light for successful courtship, some mutant strains do show behavioural differences under changing light conditions (Jacobs, 1960).

Figure 15 tabulates the results of these experiments, and the analysis of the data (Figure 15b) indicates that 65 to 75% of males generally mated under these conditions. Although there appears to be much variation, no significant differences exist between the strains 277, 55L, 55R, 55B, 55N, and CS. Line #263 is not homogenous with this set and behaves as it did in the previous mating tests with no recorded mating

Figure 15a. Mating Activity in the Dark

Strain	No. Flies Mated	Not Mated	Total	% Mated
CS	77	38	115	67.0
263	0	93	93	0.0
277	78	28	106	73.6
55L	68	39	107	63.6
55R	71	33	104	68.3
55B	54	45	99	54.5
55N	76	29	105	72.4

Figure 15b. Statistical Analysis of Figure 15a.*

i) Table 1

Strain	No. Flies Mated	Not Mated	Total
CS	77	38	115
263	0	93	93
277	78	28	106
55L	68	39	107
55R	71	33	104
55B	54	45	99
55N	76	29	105
Total	424	305	729

* based on analysis of Sokal and Rohlf (1969), pp. 599-600, 582-584.

Fig. 15b (cont'd)

ii) Significance Test

$$\text{Overall } G = 2[77\ln 77+38\ln 38+\ldots+28\ln 28-(115\ln 115+104\ln 104+\ldots+305\ln 305)+729\ln 729]$$

$$= 192.246$$

on $(a-1)(b-1) = 6$ d.f.

$p \ll .005$

mating activity in the dark is not independent of the strain of fly.

iii) Partitioning of Strain Variation: Homogeneous Groups - comparisons made between calculated and critical G value (critical G at $\alpha=.05$ level on 6 d.f., $G_{crit} = 12.592$)

largest homogeneous group:

55B - 55L - 55R - CS - 55N - 277

$$G = 2[77\ln 77+38\ln 38+\ldots+28\ln 28 - (115\ln 115+104\ln 104+\ldots+212\ln 212)+636\ln 636]$$

$$= 10.796 \text{ n.s.}$$

other comparisons of calculated and critical G may be made to confirm the following arrangement of homogeneous groups:

263 | 55B 55L 55R CS 55N 277

activity. The relative order of mating success for all lines tested was the same for both light and dark experiments: 277 > 55N > 55R > 55L > 55B > 263. However, despite suggestive differences and patterns in these results, no significant heterogeneity existed among strains (except for the slower 263 males).

Discussion

The three mutants described above are representative of the three categories of behavioural mutants outlined in the introduction. Strain 55's slow mating behaviour results from a morphological change, 263 males exhibit a general behavioural mutant phenotype, and 277 seems to be more specific in its effect.

This did not occur by design, however. Although only three strains were studied at any length, there were others that displayed altered mating activity and were not shown in Figures 3 or 4. Those with reduced mating success are listed in Appendix 4. Clearly, experiments designed to select for behavioural mutants yield many possible candidates for investigation.

Lines 55, 263 and 277 were chosen for different reasons. 263 was of interest insofar as its mating frequency was effectively zero under test conditions and it therefore displayed the most pronounced behavioural change. As stated previously, 55 was not originally identified as a morphological mutant. It was selected on the basis that it was chronologically

the first line to be categorized as a slow mater. Other slow types were set aside in favour of studying 277, the fast mating strain. It was of interest for numerous reasons. From both theoretical and practical considerations already outlined, natural selection is held to have maximized mating speed in males. The single gene lesion introduced by EMS was not expected to produce a more successful male. Indeed, the F1 male parent of the line was chosen for study precisely because it did not mate in the original thirty minute screen.

From the discussion on Drosophila mating behaviour in the introduction, various conclusions may be drawn regarding the three strains isolated in this study.

Line 263 is a severely disturbed strain and the factor responsible is sex-linked. Males are sluggish, slow to initiate courtship, and seem to have associated difficulties during courtship. Because the frequency of copulations was near zero, the sample size of the mating population was small and consequently no inferences about the distribution of copulations in time for the strain can be made.

An interesting property of 263 males was that although males did not generally mate under test conditions, there were no special problems in maintaining the line in culture. There were three reasons why this could be so: (i) The behavioural defect was such that only a low percentage ($\sim$1.4%)

of males mated in the entire population, yet their activity was sufficient to inseminate a large proportion of the females in the culture. (2) Males' (or females') mating behaviour in mass culture was not the same as in single-pair vials. This situation is known to occur in other Drosophila species, and is especially relevant for obtaining hybrid progeny when mass matings are necessary for cross-fertilization to occur (Spieth, 1952). (3) A higher proportion of males were able to mate in culture because of the effectively longer incubation time.

The first option did not seem likely because a corollary of the hypothesis is that the slow mating 263 phenotype would be under high selection pressure and possibly be lost after a few generations. Even if a single sex-linked gene was responsible for the phenotype, existing polygenic influences would be expected to modify the behaviour through selection. No experiments to distinguish between the other two possibilities were undertaken, however later observations (Dr. S.F.H. Threlkeld, private communication) indicated that given sufficient time, a higher proportion of males do achieve success in single-pair tubes.

It is of interest to consider why a longer latency period is required in this line. This could be due to a lesion that has as its primary effect a reduction in the frequency of courtship initiations or a reduction in the effectiveness of

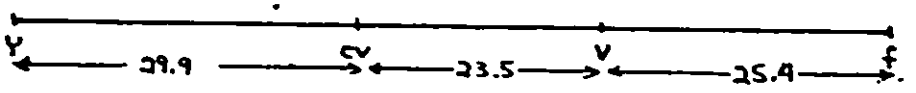
courtship itself. The first possibility represents an increased average time to the onset of courtship (that is, increased latency period in a proper sense), and the second an increase in the average duration of courtship. Undoubtedly both factors contribute to 263's behaviour. General sluggishness as seen in the locomotor tests probably manifests itself in mating activity as a reduced propensity for initiation as well as a suboptimal courtship performance. Further behavioural testing could resolve what appears to be a problem of relative contribution to the observed phenotype.

Strain 55 possesses a temperature-influenced mutation that affects wing phenotype, the severity of mutant expression being directly correlated with culture temperature. The mutation is sex-linked and maps to a position between yellow and crossveinless.

The map as constructed from the data is not, however, in agreement (apart from gene order) with the distance given in Lindsley and Grell (1968) as shown in Figure 16a. Most pronounced is the contrast between yellow-crossveinless distances (13.7 map units for Lindsley and Grell, but 29.9 m.u. from these data). Upon closer examination of the data, it appeared that the wild type allele of each marker gene was present in excess. Chi-square analysis of gene frequencies bears this out (Figure 16b). Some of this deviation for crossveinless

Figure 16a. Comparison Between Two Genetic Maps.

i) calculated from data in this study (strain 55):



ii) drawn from Lindsley and Grell (1968):

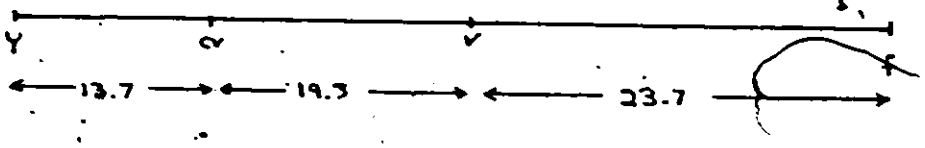


Figure 16b. Chi-square Tests on Map Data.

i) Parental Types:

	obs.	exp.
+ + + + 55	260	216.5
- - - - wild	173	216.5
Total	433	433

ii) Markers:

	Observed					Expected
phenotype	y	cv	v	f	55	(all classes)
+	554	585	578	567	540	521
-	488	457	464	475	502	521
Totals	1042					1042

iii) Chi-square:

a) parental $x^2 = \frac{(260-216.5)^2}{216.5} + \frac{(173-216.5)^2}{216.5} = 17.48$ p .001

b) yellow $x^2 = 4.18$ p .05

c) crossveinless $x^2 = 15.724$ p .001

d) vermillion $x^2 = 12.474$ p .001

e) forked $x^2 = 8.123$ p .005

f) 55 $x^2 = 1.386$ n.s.

could have been due to the difficulty in scoring flies that had an extreme 55-phenotype. Nevertheless, the fact that the departure from expectation is so great for crossveinless ($p << .001$), and because it is shown by all markers, it would seem that some systematic disturbance occurred in the mapping procedure, due perhaps to differential viability of genotypes. The effect of inflated wild allele counts upon the calculation of map distances is complex and was not fully explored. The overall result has been an only loosely applicable genetic map where only gene order can be indicated. The data do not allow for more precise location of mutant 55 except that it is in the yellow-crossveinless region of the X-chromosome.

This in itself is of some interest. Lindsley and Grell (1968) describe vesiculated (vs) as a sex-linked wing mutant first mentioned by Sturtevant in 1927 as a spontaneous mutation. A partial description of the phenotype has the "...wings warped, wrinkled, blistered, rough textured, discolored, and divergent. May overlap wild type". Vs maps to 16.3 units from the distal yellow gene and thus lies between y and cv. Both descriptions fit 55 well and raise the question whether 55 was EMS induced or spontaneous in origin.

Whether or not the newly isolated mutant is a rediscovery of vesiculated, the mating activity tests here are of equal

importance. Indeed, given the natural variation in expression of vs, it is surprising that the possibilities for using it to study the effects of wing phenotype on mating success were not recognized earlier. Other approaches, such as mutilation experiments (Ewing, 1964) and studies with more popular mutants like vestigial (Rendel, 1951), have limitations and drawbacks as others have observed. Speiss (1970), for example, suggests that many mutant studies are done with a minimum of genetic control making mutant comparisons difficult. Both vesiculated and 55 provide for the control of genetic background, present a variety of phenotypes, and avoid the problem of possible side effects of ablation. As tools for looking at morphological effects on sexual behaviour, then, these mutants would seem to be of value.

This has in fact been the case. The results of mating tests with the different 55 phenotypic classes indicate that mating success is conditioned by the mutant phenotype, with affected individuals displaying a decreased mating frequency relative to the pseudo-wild type class.

As noted above, the mating tests on strain 55 compare phenotypes rather than genotypes, the males being genetically similar and differing mainly in wing morphology. (Although the males were not isogenic, their backgrounds are identical and genetic variation is largely extraneous.) In this sense,

the 55N class elicit a 'standard response' from females to the 55 genotype bearing wild type wings. The other mating frequency curves reflect varying responses by the $\overline{XX}$ females to the other phenotypic classes.

It is important to note that if the factor(s) responsible for the initial superiority of 55N were constant in their effect, the distribution functions would be expected to maintain their relationships throughout the 35-minute test period. Obviously this is not the case; the curves are not significantly different from each other after 15 minutes. Whatever the basis for the initial lack of female response it does not operate as a constant factor, and roughly the same proportion of males mate in each subpopulation after 35 minutes.

This decay of mating disadvantage is underscored by examination of a replot of the data (Figure 8c). Here the mating frequencies are calculated relative to the number of copulations rather than the number of attempts. In this kind of presentation, one is therefore concerned with the males that mate (the mating population) in each group. Significant differences between curves in this case would mean that the temporal distributions of actual copulations are not the same. As first seen with 263D males, distributions that are dissimilar when total populations are considered may not

necessarily remain so for the mating population itself (compare Figures 10b and c). Should this be the case, it would imply that mating populations behave in similar ways; conversely, if the initial disparity persists, then their behaviours are not alike.

The results in Figure 8c do show that differences persist from 8b. Between 75-80% of 55N matings occur in the first five minutes as contrasted with 55-60% of 55L, R and B matings. Conversely, if a 55N male is not accepted by the end of five minutes, it is less likely that he will mate at all in the next 30 minutes, compared to the other phenotypic classes, since a larger proportion of the latter groups' matings occur in this later time.

Various interpretations of this result can be made in connection with the introductory remarks on Drosophila mating behaviour. Internal changes affecting male threshold levels or courtship performance could both give rise to altered mating success. Modified threshold levels for male initiation, however, might be expected to act as a constant change in the probability of initiation such that population differences would disappear upon consideration of the distribution of copulations. Moreover, since mating successs for strain 55 is correlated with mutant expression, wing morphology is presumably the only varying factor, and

not the relevant threshold levels nor the execution of courtship per se.

The fact that a higher percentage of 55N matings occur early in the test than for the other classes, while all classes eventually enjoy the same degree of success, indicates that for 55L, R, and B, there is some delay in gaining female acceptance. Given the importance of wing vibration in courtship and its suspected role in female stimulation, these results are reminiscent of the idea of courtship summation.

If wing vibration is part of the summation process, then it is expected that factors that decrease its stimulatory effectiveness would result in males having to court for a longer period of time before a female was prepared to signal acceptance. Such parameters include wing morphology (area, shape) as well as more specific aspects of vibration activity itself. For strain 55, this could explain why pseudo-wild type males are more successful in gaining female acceptance early in the test while this superiority declines to insignificant levels afterwards. Statistically, flies with reduced wing area and/or altered morphology may have to court longer to achieve female acceptance. Consequently, a substantil proportion of 55L, R, and B males who eventually mate must "wait" for summation to occur. These results and

interpretations are consistent with those of Ewing (1964) and Manning (1967), who found that wing area is an important determinant of courtship success.

Precisely because it resisted attempts at characterization in experiments that were informative about the nature of the other strains, 277 is perhaps the most interesting of the three. Since more males mated in 35 minutes than for all others, it would seem that they are in some sense "fast mating".

The precise reasons for this behaviour are not known. Given the standard females used in these experiments, higher populational success could be due to either male courtship that is more efficient at female stimulation or to an increased propensity for courtship initiation. The first case corresponds to an increased male persistence wherein more time is spent on vibration and/or licking at the expense of orientation activity. The female is expected to be stimulated to the acceptance threshold sooner than if a "regular" courtship was used. For the total male population, greater success would result from a higher proportion of successful courtships, although the proportion of courtships itself would not change. The alternative model postulates that more males are stimulated to initiate courtship at any particular time, but that

female acceptance would be achieved after a courtship of normal duration. Discrimination between these two models is best made by direct observation but the existing data offer an indication of which is the most probable.

In considering the distributions of actual copulations in the mating population of CS and 277 males (Figure 13c), differences are not statistically significant; the probabilities that mated CS and 277 males began copulation at any given time = t minutes are not statistically different. The mating populations are thus somehow behaving alike. The observed populational difference (Figure 13b) is, then, due not to a disproportionally higher frequency of 277 copulations in the early stages of the test (as was seen for 55N), but rather to the fact that mating 277 males comprise a larger fraction of the total population at all times than CS males.

As first discussed with reference to strain 55, changed courtship effectiveness would likely affect the summation process and this would be reflected in an altered mating population distribution. A constant change in the probability of courtship initiation, on the other hand, could give rise to the observed pattern of differences between 277 and CS males (Figure 13b,c).

This conclusion is readily amenable to empirical test

by measuring courtship effectiveness through recording the duration of courtship directly. Moreover, if 277 males are more easily stimulated to courtship, an additional expectation is that they begin to court sooner than CS males. By using the time to first wing vibration as a measure of this latency period, preliminary studies indicate that 277 males do begin courting earlier than CS males (Dr. S.F.H. Threlkeld, private communication).

Reduced time to male courtship corresponds to a reduced male threshold such that he more easily enters into the higher excitation levels associated with subsequent courtship activity. The finding that 277 did not differ from CS in locomotor activity supports the notion that its behaviour is altered in a more specific manner than either 263 or 55. In addition, its significant reduction in mating success during the dark tests is consistent with a model suggesting that its original superiority stems from a modified response to visual cues which play an important role in events prior to courtship.

It is important to note that the distinctions drawn here between male initiation thresholds, persistence, and courtship effectiveness are somewhat artificial. Reduced threshold is probably associated with increased persistence because the male is sexually aroused more easily. Similarly, a more

persistent male can be said to be a more efficient courter since he spends less time in non-sexual activity. The separation between these and other parameters that is made here for empirical reasons probably does not exist in the real fly. Fulker (1966) for example, has demostrated high correlations between mating speed, number of progeny, and the number of copulations.

Despite the interrelatedness of these parameters on both behavioural and genetic levels, considering them as individual components of the larger sexual system is legitimate for purposes of delineating this relationship, and also because some degree of autonomy certainly exists between them.

Although strain 277 demonstrated consistently higher mating success, the fragility of its genetic basis is revealed in the outcome of the investigative crosses to determine its mode of inheritance. 277, like 263 and 55, was founded by two outcrossings to $\overline{XX}$ (the second being a single pair mating) and then cultured inter se. Consequently, all strains were expected to have a relatively high degree of homozygosity. In crossing 277 to CS, new genetic material was supplied and heterozygosity was expected to develop. The effect of this on the behavioural phenotype was unpredictable, however in retrospect certain patterns emerged. These are discussed below.

As a phenotype, mating behaviour results from the interaction of a large number of systems in the fly, some more specific to sexual function than others. For 55 and 263, the affected genetic functions are not sex-limited; 55's altered wing morphology affects flight ability and 263 has obvious non-sexual aberrant behaviour. For the latter strain, the behavioural change was great enough so that it was observable despite an altered genetic background and thus a sex-linked inheritance pattern emerged. The observation that the predicted copulation frequency for the derived populations 263C, 263D, and 263-cross are lower than the observed values however, suggests that these outcrossings were not without their effects. In terms of the sex chromosomes, males were genotypically either 263 or CS; autosomally, the genotype was not controlled. Given the polygenic aspects of mating behaviour and the complexity of the phenotype, it is not surprising that this deviation occurs. Although 55 was not subjected to the same crosses, it is interesting to compare the original data (Figures 3 and 4) with that collected for the different phenotypes (Figure 8). The latter experiments were conducted after 55 was made homozygous through outcrossings to CS and these males were all more successful than the original line had been.

The change in mating behaviour for these two strains

is probably a general phenomenon associated with the outcrossing procedure. In other mating studies with D. melanogaster, Fulker (1966) and Parsons (1964, 1965) have demonstrated that outbred males mate more often than inbred ones, and this has been shown to be highly correlated with mating speed. Similar findings using marker karyotypes have also been reported by Speiss, Langer, and Speiss (1964) in D. pseudoobscura.

As a general occurrence, the same disturbances would be inflicted on the genetic constitution of 277 as the other two strains, except that in this case, 277's phenotype was an insufficiently drastic change from CS. The lack of resolution in the mating tests of the derived males (277-cross, 277C, 277D) appears, then, as a natural consequence of this mutant's more specific behavioural effects and the general perturbations of outcrossing observed in this study and others.

Although the results were not totally satisfactory, further comments regarding possible inheritance models may be made. The autosomal recessive model presents the most definite prediction of the relation between distributions: the parental type must be faster than all other populations. Clearly this was not found to be the case (see Figure 12).

Of the other two models, the autosomal dominant is most

like the observed result. The relative order of mating success for the sex-linked model (277 ≥ 277D ≥ 277C = 277-cross), and its predicted difference between 277 and 277C (and between 277 and 277-cross) are not borne out by experiment. The autosomal dominant model is technically deficient only in its predicted identity of 277C and 277D; both should exhibit the same relationship to CS, yet one is (and one is not) homogenous with the control males. This could be explained by the effects of outcrossing discussed above.

Closer inspection of the data reveals that other mating activity changes occurred that were not due to outcrossing. Mating frequency curves calculated from the original data (Figure 4) are invariably lower than those determined later. (This is true except for 263, though they mated at such a low frequency that comparisons were not meaningful.) For strain 55, this has been explained in part through the effects of outcrossing during the establishment of a homozygous line. Both 277 and CS males, however, show this trend as well yet neither had been exposed to this type of genetic contamination. (Note that for both sets of experiments, 277 was a more successful mater than CS, but in neither case was there a significant difference with respect to actual copulation frequency. Compare Figures 4b and 13c.)

Figure 17a. Mating Activity Data for CS Males
(Note: Set 1 experiments preceded Set 2 by approximately three months)

i) CS - CS; Set 1

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	14	6	5		1			26	38
2	8	4	3	2			1	18	20
3	8	14	6			1		29	38
4	1	5	1	1	1	4		13	15
Total	31	29	15	3	2	5	1	86	111
Cumulative frequencies (%):									
	28.0	54.1	67.5	70.3	72.1	76.6	77.5	(attempts)	
	36.0	69.7	87.1	90.6	92.9	98.7	100	(copulations)	

ii) CS - CS; Set 2

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	7	9	1					17	18
2	3	10	3	2	1			19	28
3	5	12	2	1	1			21	22
4	3	4	5					12	12
5		5	3		2			10	18
6	5	11	3	2				21	21
7	8	8	5	1		1		23	24
8	21	2						23	24
9	22	3						25	25
Total	74	64	22	6	4	1	0	171	192
Cumulative frequencies (%):									
	38.5	71.9	83.3	86.5	88.5	89.1	89.1	(attempts)	
	43.3	80.7	93.6	97.1	99.4	100	100	(copulations)	

Fig. 17a (cont'd)

iii) CS - $\overline{XX}$; Set 1 (see Fig. 3)

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	15	1	1					17	19
2	7	8	9	2	1			27	54
3	4	3						7	26
4	7	3		3		1		14	24
5	7	2	1					10	13
6	12	2	1					16	19
Total	52	19	12	5	1	1	0	91	155

Cumulative frequencies (%):

33.5	45.8	53.5	56.8	57.4	58.7	58.7	(attempts)
57.1	78.0	91.2	96.7	97.8	100	100	(copulations)

iv) CS - $\overline{XX}$; Set 2

	Time (min,)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	11	8		1			1	21	24
2	17	4	1				1	23	33
3	14	9	2	2	2			29	33
4	12	1			1	1		15	16
Total	54	22	3	3	3	1	2	88	106

Cumulative frequencies (%):

50.9	71.7	74.5	77.4	80.2	81.1	84.9	(attempts)
61.4	86.4	89.8	93.2	96.6	97.7	100	(copulations)

Figure 17b. Cumulative Distributions: CS Males Tested with CS, $\overline{XX}$ Females

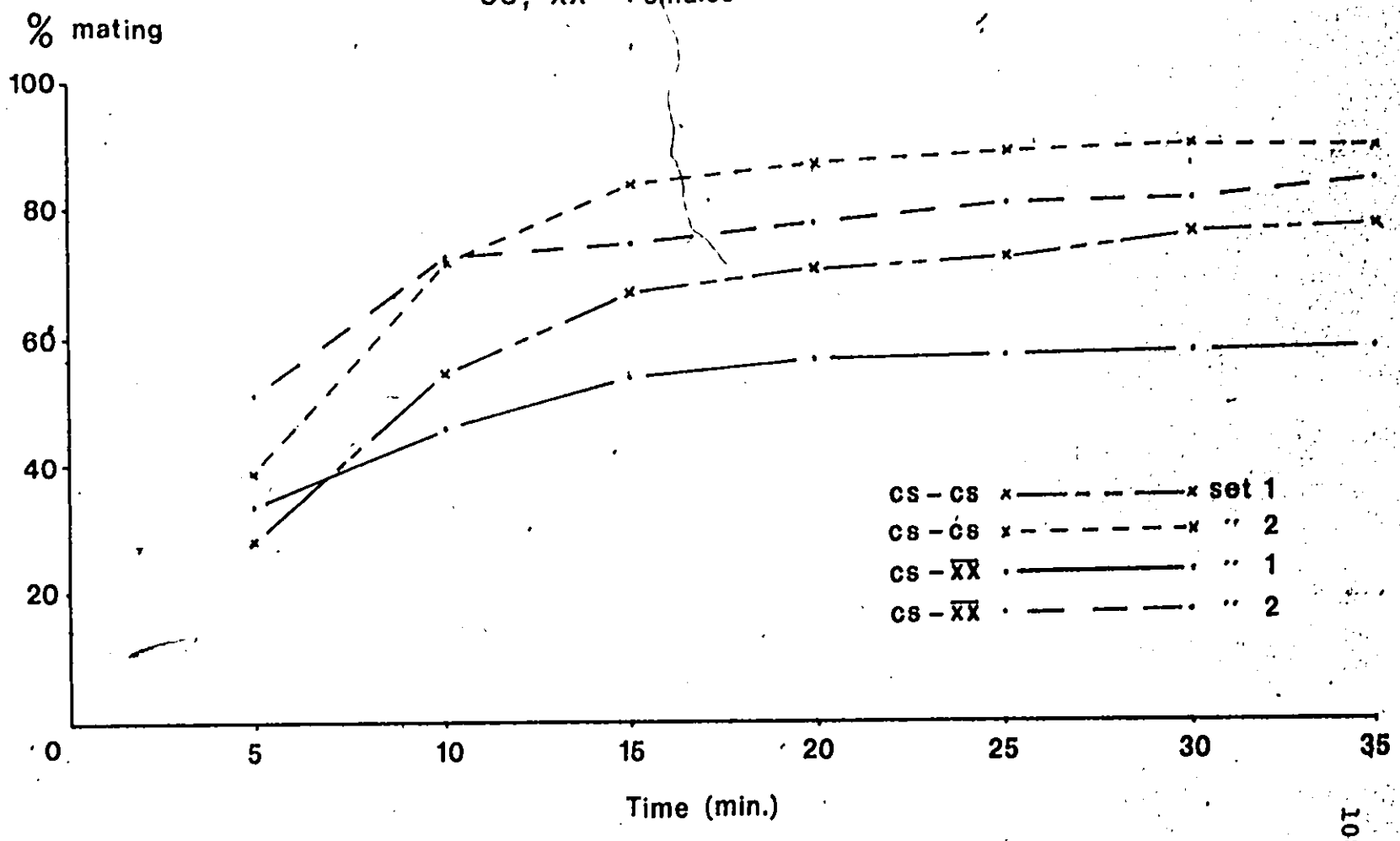
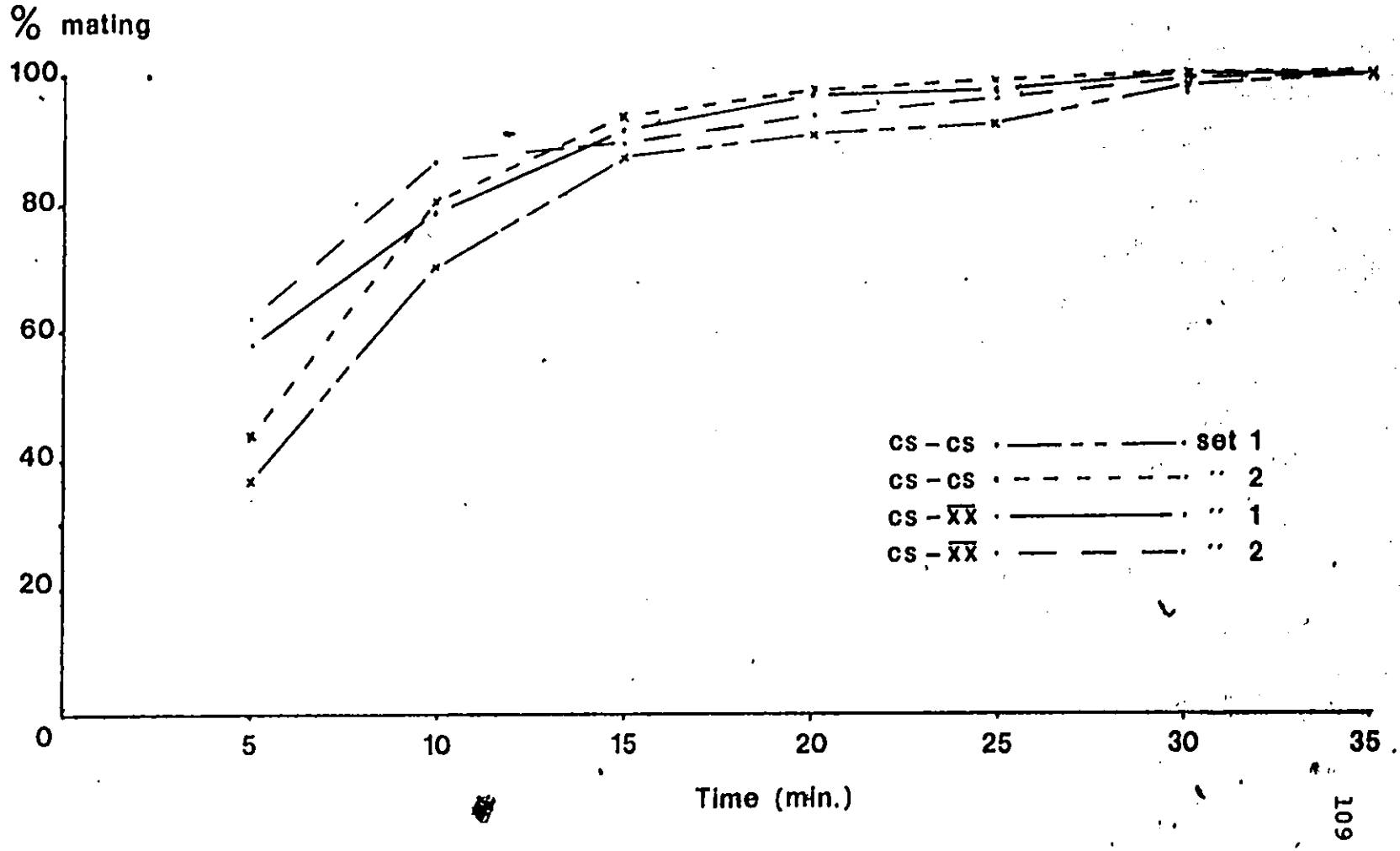


Figure 17c. Cumulative Distributions: CS Males Tested with CS, $\overline{XX}$ Females (mated pairs)



Moreover, data collected in testing CS males with CS virgin females exhibit this same tendency (Figure 17).

The appearance of increased mating success in all lines over a period of three months suggests that all strains were responding to the same influences. All cultures were maintained under the same conditions, and although the environment was controlled as much as possible, seasonal changes could have provided the impetus for the observed behaviour modification.

As a system for studying the genetics of behaviour, mating behaviour in Drosophila has many attractive properties. At the same time, there are limitations to the disturbances that this behaviour will tolerate if a line is to be maintained, and this particularly when one is interested in sex-linked mutations where it is much more difficult to preserve a behaviourally sterile recessive mutant in heterozygous form (Gill, 1963). Recent modifications to the methods used here have been directed towards isolating temperature sensitive mutants to avoid this problem (Dr. S.F.H. Threlkeld, private communication). Behaviourally sterile lines could thus be raised at a permissive temperature where reproductive activity would not be impaired.

With these three existing mutants, further genetic

techniques could be applied. Genetic mapping with strain 55 was facilitated by the presence of the morphological marker. Although it is non-morphological, 263 could be subjected to the same crosses because of its easily recognizable behavioural phenotype. As a mutant that is much more sensitive to background genetic changes, 277 could be mapped only by maintaining strict control of the genetic background. For all cases of behavioural mapping, the fact that the phenotype is best assessed in populational tests necessitates raising whole cultures of possibly recombinant males from single pair matings to $\overline{XX}$ females.

Besides these more standard genetic approaches, insight into the functional aspects of gene action may be gained through mosaic analysis. This can give information not only about structures and processes necessary for the generation of specific behaviour patterns in the adult (Hall, 1977), but also regarding its development through fate mapping (Hotta and Benzer, 1972, 1976). Here again, the use of sex-linked mating behaviour mutants presents some technical difficulties. The most convenient mosaic systems depend on the somatic loss of a sex chromosome to produce XX/XO gynandromorphs and are therefore limited to comparisons between male and female tissues. In the present case, it is of prime interest to compare mutant and wild type male

genotypes in X_1O/X_2O mosaics. Methods for generating these individuals exist - for example through the use of the 'paternal loss' mutant - but usually require more involved genetic preparation of crosses.

Lines 263 and 277 are especially attractive mutants to explore with mosaic techniques. While both mating and locomotor activity changes appear to be sex-linked in the former (and are presumably due to the same gene), these and other altered behaviours may be associated with mutant expression in different portions of the fly. Similarly, it would be of interest to investigate which structure (s) in the male are critically sensitive to the mutation carried in strain 277.

Naturally, the major goal of behaviour genetics is to understand the behaviour of an organism in genetic terms, and this can only be achieved through the two-fold method of identification and definition of behavioural parameters together with genetic analysis. The genetic tests referred to above in conjunction with additional behavioural characterization as described in the discussion of each mutant separately would serve to illuminate further the nature of these observed behavioural alterations, and, more importantly, the

normal genetic control of wild type mating behaviour in *Drosophila*.

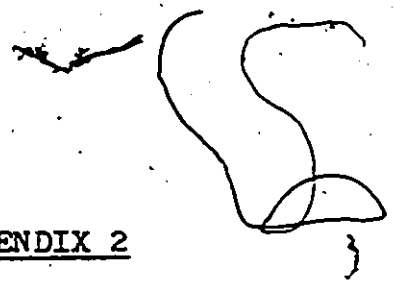
APPENDIX 1

Carpenter's Medium

Solution A:
- 900 ml. H_2O
- 15 g. agar (Bacto-agar, Difco Laboratories, Detroit, USA.)
- 100 g. sucrose
- 50 g. Brewer's Yeast
- 1 g. potassium phosphate.

Solution B:
- 200 ml. H_2O (tap distilled)
- 8 g. potassium sodium tartate
- .5 g. calcium chloride
- .5 g. sodium chloride
- .5 g. managanous chloride
- .5 g. ferrous chloride

Mix solution A and autoclave at 15 lbs. pressure for 30 minutes. Add solution B, cool and add 5.5 ml. propionic acid. Pour at 47-50° C.



APPENDIX 2

EMS solution

50 ml. distilled H_2O
5 g. sucrose
.15 ml. EMS

The above ingredients were mixed well with a magnetic stirrer. All equipment was decontaminated with .1% mercaptoacetic acid solution.

Reference: Lewis and Bacher, 1968.

APPENDIX 3

Critical Values for the Kolmogorov-Smirnov Statistic

$D = \lambda\sqrt{\frac{n_1+n_2}{n_1 n_2}}$ where: n_1, n_2 are sample sizes

λ is a constant for different levels of Type 1 error:

for	λ	α
	1.22	.1
	1.36	.05
	1.63	.01

If the greatest observed difference between two cumulative distributions exceeds D, the difference is significant at the corresponding α-level.

Entries in this table signify the group number (for ease of reference from the text), the two strains to be compared (1,2), their sample sizes ($n_1 n_2$), and the critical value (D_α) calculated for two levels of significance (α= .05,.01). The table is partitioned vertically such that the left hand side lists D values calculated for all attempts made with a given strain; the right hand side values are calculated from the total number of copulations that actually occurred. Two distributions that differ significantly have the corresponding D value underscored.

The lack of an entry reflects an insufficient sample size for 1 or both groups being compared.

Group	Strains		Attempts				Copulations			
	1	2	n_1	n_2	$D_{.05}$	$D_{.01}$	n_1	n_2	$D_{.05}$	$D_{.01}$
1	CS	45	155	91	.1796	.2153	104	75	.2060	.2469
		55		152	.1552	.1861		58	--	--
		250		124	.1639	.1964		100	.1905	.2283
		263		144	.1574	.1887		2	--	--
		271		143	.1577	.1890		114	.1844	.2210
		274		95	.1772	.2124		71	.2094	.2509
		277		105	.1719	.2060		99	.1910	.2289
2	45	55	91	152	.1803	.2160	75	58	--	--
		250		124	.1877	.2250		100	.2077	.2490
		263		144	.1821	.2183		2	--	--
		271		143	.1824	.2186		144	.2022	.2423
		274		95	.1995	.2391		71	.2252	.2699
		277		105	.1948	.2335		99	.2082	.2495
3	55	250	152	124	.1646	.1972	58	100	--	--
		263		144	.1582	.1896		2	--	--
		271		143	.1584	.1899		114	--	--
		274		95	.1779	.2132		71	--	--
		277		105	.1726	.2068		99	--	--

Group	Strains		Attempts				Copulations			
	1	2	n_1	n_2	$D_{.05}$	$D_{.01}$	n_1	n_2	$D_{.05}$	$D_{.01}$
4	250	263	124	144	.1666	.1997	100	2	--	--
		271		143	.1669	.2000		114	.1363	.2233
		274		95	.1854	.2222		71	.2111	.2530
		277		105	.1804	.2162		99	.1928	.2311
5	263	271	144	143	.1606	.1924	2	114	--	--
		274		95	.1798	.2154		71	--	--
		277		105	.1745	.2092		99	--	--
6	271	274	143	95	.1800	.2157	114	71	.2056	.2464
		277		105	.1748	.2095		99	.1863	.2239
7	274	277	95	105	.1926	.2308	71	99	.2115	.2535

Group	Strains		Attempts				Copulations			
	1	2	n_1	n_2	$D_{.05}$	$D_{.01}$	n_1	n_2	$D_{.05}$	$D_{.01}$
8	CS	55B	106	142	.1746	.2092	88	108	.1953	.2341
		55L		148	.1730	.2074		121	.1905	.2284
		55R		147	.1733	.2077		130	.1877	.2250
		55N		127	.1751	.2099		140	.1886	.2261
9	CS	263	106	144	.1741	.2086	88	2	--	--
		263-cross		125	.1796	.2152		121	.1905	.2284
		263C		102	.1886	.2261		96	.2007	.2406
		263D		111	.1847	.2214		73	.2153	.2580
10	CS	277	106	119	.1816	.2177	88	116	.1923	.2304
		277-cross		130	.1780	.2133		125	.1892	.2268
		277C		91	.1944	.2329		87	.2056	.2464
		277D		101	.1891	.2267		95	.2012	.2412

Group	Strains		Attempts				Copulations			
	1	2	n_1	n_2	$D_{.05}$	$D_{.01}$	n_1	n_2	$D_{.05}$	$D_{.01}$
11	55N	55L	140	148	.1603	.1922	127	121	.1728	.2071
		55R	140	147	.1606	.1925	127	130	.1697	.2034
		55B	140	142	.1620	.1941	127	108	.1780	.2134
	55L	55R	148	147	.1584	.1898	121	130	.1718	.2059
		55B	148	142	.1598	.1915	121	108	.1800	.2158
	55R	55B	147	142	.1600	.1918	130	108	.1771	.2122
12	263	263-cross	144	125	.1663	.1993	2	121	--	--
		263C	144	102	.1760	.2109	2	96	--	--
		263D	144	111	.1718	.2059	2	73	--	--
	263-cross	263C	125	102	.1815	.2175	121	96	.1859	.2228
		263D	125	111	.1774	.2126	121	73	.2016	.2416
	263C	263D	102	111	.1865	.2236	96	73	.2112	.2531
13	277	277-cross	119	130	.1725	.2068	116	125	.1753	.2101
		277C	119	91	.1894	.2270	116	87	.1929	.2312
		277D	119	101	.1840	.2250	116	95	.1882	.2255
	277-cross	277C	130	91	.1859	.2228	125	87	.1888	.2263
		277D	130	101	.1804	.2162	125	95	.1851	.2219
	277C	277D	91	101	.1966	.2356	87	95	.2018	.2419

APPENDIX 4

Data omitted from main thesis; other defective mating lines

Strain 32

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1			3	1		2		6	10
2		1						1	16
3	1	2	3			1		7	16
4	4		2	2	3			11	22
5		2	1		1			4	10
6	1	4	1	2	1			9	24
7	7	2	2		2			13	23
Total	13	11	12	5	7	3	0	51	121
Cumulative frequencies (%)									
	10.7	19.8	29.8	33.9	39.7	42.1	42.1	(attempts)	
	25.5	47.1	70.6	80.4	94.1	100	100	(copulations)	

Strain 257

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	2	1				2		5	27
2		1						1	8
3	2	1	1					4	9
4	1				1		2	4	13
5		2		1	2			5	24
6		1						1	8
7	1			1		1		3	21
Total	6	6	1	2	3	3	2	23	110

Cumulative frequencies (%)

5.5	10.9	11.8	13.6	16.4	19.1	20.9	(attempts)
26.1	52.2	56.5	65.2	78.3	91.3	100	(copulations)

Strain 275

	Time (min.)							Total	
Run	5	10	15	20	25	30	35	mated	tested
1	4	1	1	1				7	14
2			3					3	10
3	1	2						3	7
4			3		1			4	10
5	2	1	2	1		1		7	14
6	1	4	2					7	10
Total	8	8	11	2	1	1	0	31	65

Cumulative frequencies (%)

12.3	24.6	41.5	44.6	46.2	47.7	47.7	(attempts)
25.8	51.6	87.1	93.5	96.8	100	100	(copulations)

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