DELAYED MATING AND THE RELATIONSHIP OF RECOMBINATION TO MATERNAL AGE IN *DROSOPHILA MELANOGASTER*[1]

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UNDER ordinary circumstances a *Drosophila melanogaster* female mates a few hours after emerging from the pupa case, and approximately one day after her emergence she begins laying fertilized eggs. The recombination values then obtained vary with the maternal age at which the eggs are deposited, and the degree of the variation depends upon the chromosome region under observation. It appears reasonable to believe that these recombination effects are due primarily to the physiological condition of the mother at the time the chromosomes are undergoing crossing over in the particular oocytes which are concerned. Of definite interest in this connection is the common observation that the markedly heterochromatic regions about centromeres are quite sensitive in recombination behavior to these physiological changes with age.

Now it can happen that a female does not mate immediately on emergence, and the laying of eggs is delayed. Various factors will affect the time of her mating—these include: the vitality of male or female, the age of the male, sexual preferences between different mutant types (as in the cross of non-yellow female by yellow male), the number of males present (as in individual matings), such cultural accidents as temporary trapping of either sex, and so on. If the number of delayed matings becomes sufficient (and under some circumstances this sufficient number may be very small), inclusion of data from late-mating females with data from early-mating females of the same age is expected to distort those recombination values which are sensitive to age, and to affect interpretation of the results.

Storage of oocytes which are mature except for the maturation divisions themselves is known to occur in Drosophila virgins from studies of egg laying and from direct cytological observation (LAURINAT 1931; PATTERSON, BREWSTER, and WINCHESTER 1932; KING, RUBINSON, and SMITH 1956; DAVID 1963; and others). To clarify effects of the situation in which the early eggs of a virgin have undergone a normally timed chromosome exchange at or near pachytene and are stored within the mother's body as oocytes, let us momentarily ignore other complications. After the delayed mating these eggs are fertilized; they are soon laid (at an abnormally late maternal age), and they develop. On the basis of such a series of events the proportion of crossover adults obtained from these particular eggs

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will be determined at the usual maternal age; but the date of deposition of the eggs, by means of which the age change is in fact experimentally measured, has been shifted. And *ipso facto* age change in recombination for sensitive regions is altered.

Actually a number of other factors must simultaneously be concerned in the production of net recombination values computed from the F_1 adults. Thus under certain conditions unfertilized eggs are laid in large numbers before mating occurs; such eggs do not develop. This elimination, selective with regard to maternal age, will independently distort recombination values. Also if the virginity continues for a considerable period it seems possible that there might be another elimination effect due to degeneration of oocytes within the mother's body—and PATTERSON, BREWSTER and WINCHESTER (1932) believe that their observations on egg laying provide inferential evidence for this. It is further conceivable that retained eggs which are only slightly affected by degenerative change may still be laid after mating occurs, and that although they may (or may not) have been fertilized they are subsequently unable to complete their development.

It is obvious that if change from control egg mortality rates and from control rates of egg production and egg deposition are properly taken into account, we can expect to reach conclusions which are more directly related to the actual process of crossing over than is otherwise the case. This is especially true when the experimental agent itself might reasonably be under suspicion of producing such secondary change, as, for example, if chemicals known to delay cell division are used. Thus it is desirable to determine to what extent normal age change in recombination is distorted when such rates have been consciously changed, as when a female is held virgin for a prolonged period after emergence.

Data measuring this specific effect of delayed mating have apparently not previously been reported, but the possibility of a similar effect was mentioned by BRIDGES in 1929 in reviewing results published by BERGNER (1928) on recombination observed after lengthened maternal development. (The lengthening was brought about by exposure at various stages to alcohol, formalin, carbon dioxide, semistarvation diets, and so on.) An observation of SCHULTZ and BRIDGES also is relevant which points out (MORGAN, BRIDGES and SCHULTZ 1931) that egg laying curves and crossing over curves (for sensitive regions) exhibit reciprocal shapes. Indeed they studied the relations of the positions of the minima in egg laying and the maxima in crossing over with the idea (as DR. SCHULTZ informs me) that the rate of egg laying might influence the amount of time available for the synaptic process—but they could draw no definite conclusions from their data. The present data apparently do not support this idea. Finally the results of BROWN and HANNAH (1952) should be mentioned which show an effect of age at mating on the proportion of gynandromorph offspring from mothers heterozygous for a ring X chromosome. They concluded that this is due to elimination of the ring X. The age range at which this virginity was found effective (that is, 7 to 15 days) is, as will be seen, suggestively like that at which marked mortality effects first become evident in the data of the present experiment (that is, after six days of virginity).

THE EXPERIMENT

Material: The region from scarlet to Stubble about the spindle fibre of chromosome 3 was chosen for testing, since here crossing over is quite sensitive to age (BRIDGES 1927, 1929). (BRIDGES and BREHME [1944] give the standard locus of scarlet, *st*, as 44.0, and that of Stubble, *Sb*, as 58.2.) Comparable *st Sb*/+ virgin females were derived and were placed in 1 × 4 inch shell vials (one female to a vial), these vials having been provided with the yeasted cornmeal-agar-molasses medium of this laboratory. The vials were then separated into three groups. To each vial of one group were immediately added ten scarlet males; the parents were subsequently transferred at 2-day intervals to quarter-pint culture bottles, five fresh males being added to each new bottle at each transfer. The parents of this first group were discarded when the mothers reached 13 days of age. Females of the second group were held 4 days without mating, and were then also transferred with males each 2 days until some of the females (but not all of them) were 17 days old; thus data were available here, as they were for the first group, for 12 days of laying after mating. The third group was of females held virgin 6 days; after the isolation period these females were similarly mated and transferred—some of the mothers in this case were also allowed 12 days of laying after mating, reaching the age of 19 days. All vials and bottles were kept at 25.0° ± 0.5°C. The mothers in all three groups began, with few exceptions, to lay fertilized eggs soon after they were exposed to males. The precaution was taken of excluding those few mothers which were not productive immediately on the addition of males, since data from such females would complicate the interpretation. (Of 13 mothers exposed to males immediately after emergence, two did not produce offspring in the first set of vials; 1 of 19 mothers held virgin 4 days did not give F_1 when males were first added—and so also 3 of 17 of the mothers held virgin 6 days.)

The egg counts: Before proper comparisons can be made of the recombination data emerging from the three types of mother some information should be available concerning their egg laying and egg mortality. Of obvious importance are any eggs which are eliminated, since they do not enter into recombination calculations based upon adults. Simple inspection through the glass walls of the vials showed that while the mated females laid copiously, only some of the females held virgin gave eggs before they were mated. (These unfertilized eggs, of course, do not hatch.) Thus the 11 mothers which mated almost immediately laid eggs in the vials producing an average of 55.4 adult offspring per female for the first 2 days (Table 1). This is to be regarded as a good yield for this first period; it is therefore certain that conditions in the vials were conducive to the laying of fertilized eggs. But these same conditions were not conducive to the laying of unfertilized eggs by the 4-day virgins—only 1 of the 18 usable females held virgin 4 days (some 5%) gave any eggs during the 4-day period, and this female gave only two such eggs. However 5 of the 17 females held virgin 6 days (that is 29%) produced eggs during the period of virginity. There was some difficulty in counting these latter eggs on the cornmeal medium through the glass walls of the vials; the approximate number in vials which showed eggs varied between 4 and 16, the total reaching some 73 (or possibly a few more). This would mean an average of about 15 eggs per *laying* virgin—or in terms of all females held virgin 6 days, an average of about 4.4 eggs per female.

For data on egg mortality, mated *st Sb*/+ females were allowed to lay on egg trays, these trays having been provided with the agar-molasses-yeast-medium in use in this laboratory for egg collection (TRAVAGLINI 1956—and adapted for measuring mortality rates by REDFIELD 1957). Before mating, the 4-day and 6-day virgins were kept in vials containing the ordinary yeasted cornmeal medium in order that their egg laying behavior would be comparable with that of the similar 4-day and 6-day virgins from which the recombination data were obtained. (Virgin females lay larger numbers of eggs on the medium lacking cornmeal; but after mating, females lay equally well on either medium.) The control mothers, exposed to males immediately after emergence, laid practically no eggs on the trays on the first day (less than 0.3 egg per mother); but they laid eggs in reasonably large numbers on the second day. A second examination of the eggs some 30 hours after the first counts, showed that 9.4% remained unhatched. (This value is for the first 2 days combined.) Afterwards (for the next 6 days) egg mortality for these females

averaged a relatively stable 7.8%. During their period of virginity the 4-day virgins in these measurements produced only a negligible number of eggs in the vials—as had also the 4-day virgins used to obtain the recombination values of Table 1. The 6-day virgins here did give some unfertilized eggs in the vials; these were similar in number to the total, previously described, of 4.4 eggs per female for the period of virginity of the 6-day virgins providing the data of Table 1. For the mortality counts the 4-day virgins produced a large number of eggs on the fifth day, 10.3% of which remained unhatched—this value is only slightly greater than the 9.4% exhibited on the first 2 days by eggs from the control females. Later mortality rates (measured for one week) for these 4-day virgins averaged 7.2%, approximately the same as the 7.8% for control mothers. In contrast, the 6-day virgins which laid a comparably large number of eggs on the seventh day, gave a high mortality rate of 31.0% among these eggs—the difference between this rate and the corresponding rate for the first eggs laid after mating by either control mothers or by 4-day virgins is just over 20. Values subsequently obtained (for one week from the 6-day virgins) were reasonably stable and only slightly higher than comparable values for the other two types of mother, averaging some 8.7% instead of 7.8 or 7.2%.

These results (with one exception to be discussed in the next paragraph) are similar to the previous results of LAURINAT (1931) and PATTERSON, BREWSTER, and WINCHESTER (1932) on egg laying and egg mortality, in spite of the fact that the earlier studies were conducted with different stocks and under different cultural conditions. Fortunately for our present purposes this paper of PATTERSON *et al.*, as well as one of KING, RUBINSON, and SMITH (1956), demonstrated by means of dissection that the numbers of mature oocytes within the ovarioles of virgins and mated females are very different. For mated laying females the former paper reports 2.6 such oocytes per female on the first day, and 10.5 on each of the next five days. Virgin females deposited practically no eggs; but counts of internal oocytes for the first six days were in order: 0.7, 34.7, 56.2, 53.8, 76.5, and 76.7 per female. Thus there is no doubt that mature oocytes are stored internally in considerable numbers by virgins. GREGG and DAY (1963) have briefly reported variation, depending upon genetic constitution, in the number of such mature oocytes in ovarioles of 4-day virgins. The exact numbers of stored eggs in the present *st Sb/+* females are not known. But since these females presumably are similar in their capacity for egg production to the females used by PATTERSON, BREWSTER, and WINCHESTER it seems likely that the oocyte storage also is similar in the two experiments.

The one significant detail in which the present egg mortality rates differ from those found by PATTERSON *et al.* appears in comparisons of eggs giving rise to first broods. They found that eggs deposited on the first day after mating, by females which had been held virgin, showed some 20% more egg mortality than eggs of first broods from females mated immediately on emergence. And it was immaterial how long the virginity lasted in their females, at least for any period up to nine days. Subsequent broods exhibited only negligible differences in mortality rates. But in the present experiment egg mortality of first broods does depend upon the length of the period of virginity. For although the 20% difference similarly holds here for 6-day virgins compared with control females; it does not hold for the 4-day virgins, whose egg mortality rates are almost the same as those for control mothers. Thus the lethal effect, detected in these 6-day virgins and presumably acting on the oldest retained eggs, is initiated at a definite maternal age. After mating, however, the egg mortality shows quick and practically complete recovery to the normal rate. One is reminded of suggestions (WHEELER 1947; CHEN and DIEM 1961) that acceleration after mating, in egg production and deposition, is brought about through indirect reaction to the paragonial secretions of the male, or perhaps occurs as the result of direct reaction to courtship and mating.

In any case, among eggs laid after mating on the seventh day by the 6-day virgins there is either: (1) a relatively very high proportion of unfertilized eggs which had undergone considerable degenerative change and could not be fertilized, or possibly were quite normal but were laid too rapidly to be fertilized; or (2) a very large proportion of eggs slightly defective after retention, which were fertilized, but degenerated completely on being laid; or, perhaps more likely, there is (3) a combination of proportions of these degenerating fertilized and unfertilized eggs.

A complication frequently encountered in work involving change with maternal age arises in these egg counts; it is caused by use of a transfer period which is unsuitably long and which leads to the pooling of nonhomogeneous data. As stated, egg mortality for the seventh day of the 6-day virgins was 31.0%; on the eighth day for the same females it dropped to a value of 9.2%. Combining data for these two days produces a value of 16.3. Since elimination of eggs from the first day after mating accounts disproportionately for this value from the combined data (229 eggs dead for the first day after mating as opposed to 140 for the second day) it is obvious that this pooling markedly distorts the mortality picture. Furthermore if crossing over has been quite different among eggs laid on the two days the recombination effect will necessarily also be distorted by the pooling. Such probably has to some degree modified the recombination value obtained for the first brood of the 6-day virgins.

For use now in considering the material of Table 1 we may summarize the following points. It is clear that virgins tend to store mature eggs; and that mating does speed up the production and deposition of eggs. Mating, however, is not a necessary condition for the laying of eggs. The number of eggs stored by virgins plus the number of unfertilized eggs they lay is, during the period of virginity, definitely lower than the number of eggs laid during the corresponding age period by mated females. Under the circumstances of the present experiment a female held unmated four days lays, for all practical purposes, no eggs before she mates; but a considerable proportion of females unmated six days will, by the end of the period of virginity, have laid some eggs. Special care was taken to keep cultural conditions comparable for the three types of mother. Finally egg mortality exhibited just after mating is relatively very high for the females held virgin six days.

The recombination results: Table 1 gives the raw data for all classes of offspring for the three types of mother and for each category of maternal age; it shows also the average N per mother, the average percent of total productivity per mother (that is the N per mother for a given transfer divided by the grand total N per mother), and the recombination for the *st-Sb* region. Viability imbalances are not appreciable. Thus for mothers mated immediately *st* was found in 49.5% of all F_1, and *Sb* in 48.7% (where expectation, of course, is 50.0). Corresponding values for mothers held virgin 4 days were 50.6 for *st*, and 50.4 for *Sb*; for the 6-day virgins they were 49.6 for both *st* and *Sb*. For that transfer which probably involved the least favorable cultural conditions, namely for the vials in which mothers (mated immediately) laid eggs on the first two days, the values were still good: they were 47.8 for *st* and 49.9 for *Sb*, although N for this transfer (609) was not as high as might be desired.

Recombination values for the given age categories disclose differences between the types of mother. The general pattern of effects is most easily followed in the graphs of Figure 1. A fall and rise in value, as is commonly observed for recombination in this region (BRIDGES 1929, and many others), is shown by all three curves. (The secondary rise is greater than is ordinarily found, but it is shown consistently by all three types of mother. It depends, no doubt, upon the particular genetic modifiers present, these being the same for all the mothers.) The time at which the early minimum appears, that is the materal age at which net recombination ceases to fall and begins to rise, differs according to the time of mating.

TABLE 1

Effects on recombination of delayed mating in st Sb/+ *mothers*

	Maternal age in days						
	1+2	3+4	5+6	7+8	9+10	11+12	Total
Mothers mated immediately after emergence							
st Sb F_1	244	721	738	716	699	568	3686
+ F_1	258	800	745	764	732	543	3842
st F_1	47	119	95	118	147	141	667
Sb F_1	60	98	90	112	126	113	599
N	609	1738	1668	1710	1704	1365	8794
N per mother	55.4	158.0	151.6	155.4	154.9	124.1	799.4
Percent of total productivity	6.9	19.8	19.0	19.4	19.4	15.5	100.0
st-Sb recombination	17.6	12.5	11.1	13.4	16.0	18.6	14.4
Mothers held virgin 4 days after emergence							
	5+6	7+8	9+10	11+12	13+14	15+16	
st Sb F_1	787	1193	1010	565	217	146	3918
+ F_1	793	1159	1035	520	192	127	3826
st F_1	156	114	172	120	46	32	640
Sb F_1	143	141	159	119	35	21	618
N	1879	2607	2376	1324	490	326	9002
N per mother	104.2	153.3	139.4	132.4	98.0	81.5	708.8
Percent of total productivity	14.7	21.6	19.7	18.7	13.8	11.5	100.0
st-Sb recombination	15.9	9.8	13.9	18.0	16.5	16.2	14.0 (14.7)*
Mothers held virgin 6 days after emergence							
	7+8	9+10	11+12	13+14	15+16	17+18	
st Sb F_1	435	824	904	409	250	302	3124
+ F_1	454	871	882	440	279	254	3180
st F_1	86	94	103	106	66	62	517
Sb F_1	73	105	146	88	48	55	515
N	1048	1894	2035	1043	643	673	7336
N per mother	74.8	145.7	156.5	149.0	128.6	134.6	789.2
Percent of total productivity	9.5	18.5	19.8	18.9	16.3	17.0	100.0
st-Sb recombination	15.2	10.5	12.2	18.6	17.7	17.4	14.1 (15.2)*

* Corrected for variation in the number of mothers used—see text.

It is immediately obvious also that the curves for the two types of delayed matings resemble each other in shape; that is their differences depend to a considerable degree upon their placement along the axis of abscissae. In addition to these differences depending upon position, are differences in shape—especially those shown by the curve for the mothers mated immediately when it is compared with either curve for mothers held virgin. The former is wider, and takes all of 12 days after mating to reach the high value. When egg laying begins, this curve reaches its first minimum more slowly, requiring three transfers instead of two; and then it more slowly ascends to its high value, taking three additional transfers instead of two. Thus this cycle, measured from the time of mating to the time of the greatest value attained, takes four days longer for completion in the case of

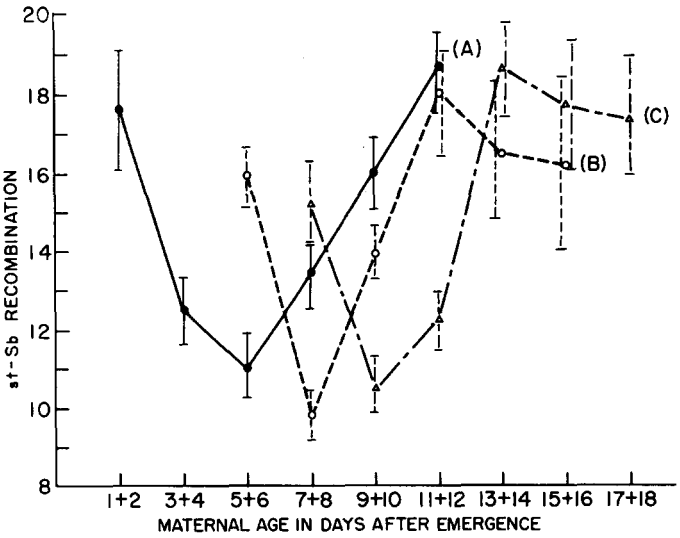

FIGURE 1.—Effects of maternal age on net recombination at the spindle fibre region of chromosome 3 for *st Sb*/+ females which were: (A) mated on emergence; (B) held virgin 4 days before mating; and (C) held virgin 6 days before mating. The vertical bar for each value represents ± SE.

females mated immediately on emergence than it does for females held virgin either four days or six days.

The total recombination values for the 12-day laying periods may be considered briefly. As they stand the raw data produce the following total values: 14.4 with a standard error of 0.37 for the control mothers, 14.0 ± 0.36 for the 4-day virgins, and 14.1 ± 0.41 for the 6-day virgins. It is worth noting that if one is at all to compare such total values, those here for virgins must be corrected since not all mothers which had been held virgin were carried through an entire 12-day period after mating. Computations based upon N's per mother rather than upon gross total N's obtained, provide corrected values of 14.7 for the 4-day virgins and of 15.2 for the 6-day virgins. The degree of change is not of interest, since actual differences here are largely obscured by the pooling of transfers. Nevertheless the existence itself of the differences demonstrates that recombination distortion is to be expected for a sensitive region if during the experiment some mothers die, or if for any other reason some of them partially or completely stop laying eggs—that is, of course, providing the given period of pooling involves age change.

A more realistic picture of what is happending is provided by detailed comparison of results from the 2-day transfers. It seems desirable to consider these results from two points of reference, namely emergence and mating.

Comparisons at corresponding 2-day periods after emergence: Even cursory examination of the curves of Figure 1 suggests that there are differences of considerable significance between recombination values for the three types of mother at specific maternal ages after emergence. Thus at age 5 + 6 days mothers mated

immediately and 4-day virgins provide a difference in recombination of 4.8 ± 1.14. At age 7 + 8 days the difference is 3.6 ± 1.01. For the two later transfers common to these two types of mother, that is for days 9 + 10 and for 11 + 12, the differences show less significance (2.1 ± 1.14 and 2.4 ± 2.40 respectively). Thus there is convergence of recombination values with maternal age—that is recovery of the mothers held virgin toward normal recombination behavior.

Similar comparison of mothers mated early and mothers held virgin 6 days shows very significant differences at ages 9 + 10 days and 11 + 12 days, amounting respectively to 5.5 ± 1.13 and 6.4 ± 1.28. But for the first transfer concerned (at age 7 + 8 days) the difference, 1.8 ± 1.38, is not significant. In contrast to 4-day virgins, these 6-day virgins do not show terminal recovery by age 12 days, although there is sudden recovery to the high value in the succeeding transfer.

Significant differences are also detected when the 4-day virgins are compared with the 6-day virgins for the age ranges 7 + 8 days, 9 + 10 days, and 11 + 12 days. The differences are, in the same order as these maternal age categories: 5.4 ± 1.25, 3.4 ± 1.00, and 5.8 ± 1.28. The last two comparable transfers (13 + 14 days and 15 + 16 days) show close approach of values; the differences amount in order of age to 2.1 ± 1.61 and 1.5 ± 2.68—that is, there is a sudden tendency of the two types of virgin to approach similar (normal) behavior.

A measure of the degree of similarity, if any, which exists between the three complete series of values for the three types of mother is possible by use of chi square tests. Thus on comparing the four transfers common to mothers mated early and the 4-day virgins it is found that for $\Sigma \chi^2$ the corresponding value of P is less than 0.001, indicating no perceptible resemblance in statistical terms. However it is of interest that χ^2 values for the four transfers taken separately correspond to gradually increasing values of P from $P < 0.001$ to the final $0.60 < P < 0.70$.

Chi-square tests for mothers mated early versus 6-day virgins show no resemblance for the series of three transfers involved here—P remains less than 0.001.

For 4-day virgins versus 6-day virgins the two series of five comparable values also give $P < 0.001$, still indicating negligible resemblance in total behavior. However the gradual recovery toward like behavior in later transfers is indicated by increasing values of P: thus for age 13 + 14 days $0.20 < P < 0.30$, and for age 15 + 16 days $0.50 < P < 0.60$.

Comparisons at corresponding periods after mating: Now that differences in recombination exhibited by the three types of mother at the same age have been shown to be significant, it is of further interest to determine the significance of differences when the 2-day periods are counted after mating rather than after emergence. Differences in the curves of Figure 1 do depend to a considerable degree upon position along the axis of abscissae—thus if other differences than those of position were found to be statistically insignificant we might be inclined to explain the observed effects in terms of a simple delay, equal to the period of virginity, in given batches of eggs.

On comparing values for the same single periods after mating for 4-day virgins and for control females, only the fourth such period gives a really significant

difference—this amounts to 4.6 ± 1.34. However we are primarily concerned here with the degree of similarity between the recombination values considered as two comparable *series*; and for this chi square tests are especially useful. For the six transfers $\Sigma \chi^2$ corresponds to a very low value of P, less than 0.001. Nevertheless for the last two transfers P is appreciable, $0.50 < P < 0.60$; thus there is considerable recovery as compared with the first four transfers where P is much less than 0.001. Similar comparison of 6-day virgins and control females shows also a significant difference for the single fourth period after mating, amounting to 5.2 ± 1.46. And the chi square test in this case for the two series of six laying periods also similarly gives $P < 0.001$. For these 6-day virgins somewhat less approach of the last two transfers to normal behavior is indicated than for 4-day virgins. for now $0.30 < P < 0.40$. It should be noted that the extremely low values of P, both less than 0.001, for the 12 days' control values as a series versus the 12 days' values for either type of virgin, are not consistent with an explanation in terms of simple retarded appearance of given batches of eggs. This, of course, is in accord with the evidence concerning changes after prolonged virginity in both egg mortality and in the production (as opposed to the deposition) of the eggs.

A similar comparison of such corresponding periods after mating for 4-day virgins and 6-day virgins shows a greater tendency for differences to disappear. Thus the largest single difference found amounts only to 1.7 ± 1.01. And values of P from chi square tests are, compared with those given above, high. For the complete series of six transfers $0.10 < P < 0.20$. And for the last three transfers P attains the very high value of $0.80 < P < 0.90$; that is the three terminal values for the two types of virgin are, as series, alike to an extremely significant degree.

We may now consider the bearing of the comparison of recombination values at corresponding periods after mating, on the hypothesis of SCHULTZ and BRIDGES which relates age change in recombination to the amount of time available for chromosome exchange. Data for 4-day virgins are more useful here than those from 6-day virgins because the alteration in recombination which the former show is not complicated by elimination through markedly increased egg mortality. If, then, the observed delay in egg deposition by 4-day virgins did involve a considerable lengthening of the time during which crossing over is possible, one would expect in early broods *higher* recombination values than for control mothers. However values for the first two productive transfers of 4-day virgins are not higher than control values—they are slightly lower. (The difference for the initial brood amounts to 1.7 ± 1.75; for the second brood it is 2.7 ± 0.98.) The nonappearance of increase in the values from virgins suggests that the known delay in deposition of early eggs does not involve appreciable lengthening of the actual period of chromosome exchange. The delay apparently operates at a later stage in development. And since the observed storage of mature oocytes in such virgins is less than egg deposition by control mated females (PATTERSON, BREWSTER. and WINCHESTER 1932; KING, RUBINSON, and SMITH 1956) delay at egg stages intermediate between crossing over and maturity is indicated.

Rates of change in egg laying are also significant in connection with the hy-

pothesis of SCHULTZ and BRIDGES. By combining observed egg mortality for the three types of mother with the numbers per mother of adults from similar mothers (of Table 1), approximate rates of egg laying can easily be computed. The resulting variation with maternal age, in the number of eggs laid per female is shown by the curves of Figure 2. There is, of course, for each type of mother an initial low deposition of eggs and a corresponding high recombination value derived from the F_1 adults. But subsequent changes in these values do not consistently follow this inverse relationship, as comparison of Figures 1 and 2 readily shows. The details need not be given. Nevertheless it should be noted that if prolonged synapsis were a major influence in the production of the differences in recombination, delayed mating of the 4-day virgins would be expected to give an increased lag between the time of synaptic exchange and the appearance of the resulting recombination change. This would be evidenced by an increase for females held virgin, compared with control females, in the period between the appearance of the recombination minimum and the egg laying maximum. Such is not found. The delayed matings, particularly those of the 4-day virgins, provide tentative evidence against the alteration expected, on this hypothesis, in the period of lag.

DISCUSSION

The results of the experiment show that recombination values for a sensitive region depend not only upon the maternal age at which the eggs are deposited, but they depend to a marked degree upon the age at which the mothers mate. Delay in mating is accompanied by changes in the normal rates of egg production, egg deposition, and egg mortality; and these changes alter the proportions of

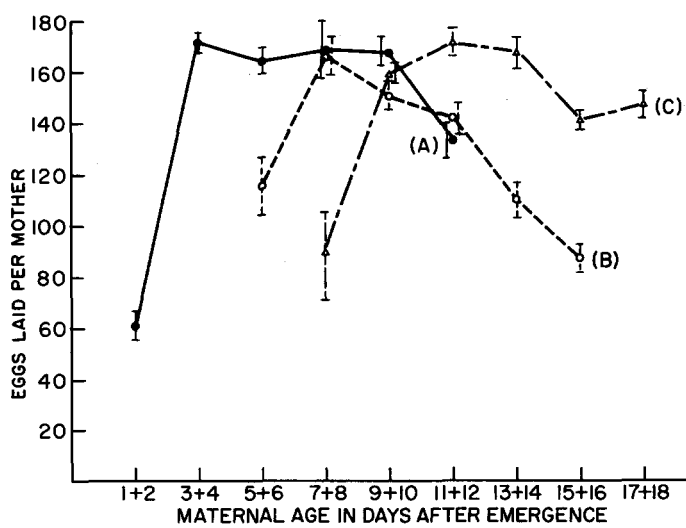

FIGURE 2.—Effects of maternal age on the rate of egg laying for *st Sb*/+ females which were: (A) mated on emergence, (B) held virgin 4 days before mating; and (C) held virgin 6 days before mating. The vertical bar for each value represents ± SE.

recombinants which appear. It follows that data on recombination will be more closely related to the actual process of crossing over when experimental accidents or experimental agents which affect these secondary processes are taken into proper account. Since in the present experiment delayed mating has consciously been used as an agent responsible for such retarding and mortality effects, we may now ask what conclusions can be derived from the results concerning the actual weights of such influences.

The egg mortality effects are definite; they are of importance in considering recombination data from the 6-day virgins, particularly data supplied by the initial productive broods. Fortunately such increased embryonic mortality is not a complicating factor in comparing the 4-day virgins with control females—thus isolation occurs here of the retardation effects dependent upon storage and delayed production. It was found that while the 4-day virgins lay only 0.11 egg per mother during their period of virginity, control mated mothers during the same age period of 4 days lay 232 eggs per female. And since, as has already been pointed out, there are grounds for believing these *st Sb*/+ virgins store approximately the same number of mature oocytes as did the 4-day virgins in the cross of PATTERSON, BREWSTER, and WINCHESTER (1932)—namely about 54—it is very likely that some 54 of the 232 eggs constituting the normal yield here for the first 4 days, that is 23% of this yield, are in storage as mature oocytes in ovarioles of the present 4-day virgins. This means that 178, or 77%, are at this age in these virgins delayed at an earlier stage.

Computations involving the data from the *st Sb*/+ females alone (without recourse to results from other crosses) will further clarify this retardation of egg production. Let us assume for the moment that the recombination value observed for the first brood from 4-day virgins is due to elimination of earliest oocytes. Table 1 shows that up to 7 days 365.0 F_1 adults were obtained per control mother; with the observed egg mortality this implies the laying of some 396.0 eggs. For 4-day virgins 104.2 F_1 adults were produced per mother to age 7 days, corresponding to about 114.5 eggs per mother—that is, 71% of the eggs deposited during the same time by the control mother are missing. If this absence depended upon elimination of the oldest oocytes the missing eggs would include: all 60.9 per control mother laid on days 1 + 2, all 171.0 eggs laid on days 3 + 4, and 50.4 of 164.1 eggs laid on days 5 + 6. Expected recombination would then be slightly more than 11.1, this value being that observed for control mothers for days 5 + 6 (the slight increase being due to the upward trend for recombination values at this stage). But net recombination for the first brood from 4-day virgins is 15.9—the difference is just under 4.8; it is significant for it has a standard error of about 1.00. Thus elimination of the earliest oocytes will not account for the recombination value actually obtained. The early highly recombinant gametes must persist in this first brood in very nearly the same proportion as in the first brood of control mothers. Retardation of production of somewhat later germ cells is indicated; and in view of previous considerations this apparently occurs at a developmental stage of the germ cells intermediate between chromosome exchange and oocyte maturity. In physiological terms the retardation may be described as due to a

feedback mechanism dependent upon the state of sustained virginity as such.

Similar comparison using 6-day virgins provides a much smaller difference between the recombination value expected on the basis of elimination, and the observed net recombination value for the first brood. Thus to the ninth day of age a control mother has laid 564.1 eggs—a 6-day virgin only 93.8 eggs (4.4 unfertilized eggs during the 6 days of virginity plus 89.4 eggs as computed from the F_1 count of the first brood and the observed egg mortality). The difference, 470.3, represents 83.4% of the eggs laid by the control female. In accordance with the assumption to be tested, the missing eggs then include all those laid to age 7 days by the control mother, namely 390.6, and 74.3 also (44.1%) of those laid on days 7 + 8. Expected net recombination after this elimination would be somewhat greater than the observed control value, 13.4, for this latter age (i.e. greater since the trend is upward). The observed value for 6-day virgins is 15.2; the difference in this case is less than 1.8, and is not statistically significant. Elimination of the oldest oocytes may then be expected to be a factor of marked weight for the 6-day virgins. This is not surprising considering the following two facts demonstrated for the 6-day virgins (but not found for 4-day virgins): (1) the deposition during the period of virginity of a number of unfertilized eggs; and especially (2) the extremely high egg mortality among eggs giving the first brood. It is further possible that after such prolonged virginity there is some slight disintegration of eggs within the mothers' bodies, as was believed by PATTERSON *et al.* (1932) to be the case. Such influences, of course, do not exclude the operation in the 6-day virgins of retardation of egg production as an important factor.

Since 83.4% of the expected eggs are missing from the first brood of 6-day virgins, it may appear odd that the remaining eggs give as high a recombination value as they do. However control mothers at the age of 7 and 8 days are laying eggs which show a rapidly increasing proportion of crossovers. So it may well be that after the prolonged virginity many or all of the earliest oocytes, in addition to intermediate ones also, do not appear as F_1 adults—still after these particular gametes are out of commission, the resulting net recombination attains an initial high value due primarily to the physiological condition of the mother with age at the time the surviving eggs had undergone chromosome exchange.

A laying period of some 3 to 6 days, or longer, is frequently used in Drosophila work since this is convenient, and for such cultures the time of the original mating is usually unknown. It becomes increasingly clear that to insure a homogeneous population of recombination values it is advisable to use suitably short transfer periods, and (in order to detect individual differences) separate matings of single mothers. Theoretically, of course, complications due to time pooling of data appear to some degree for even a very short laying period when any region under study exhibits, within the period, any modification in recombination with maternal age.

The curves of Figure 1 show an obvious effect of virginity on the amount of time required, after the mating occurs, for the first recombination cycle. This is true when either of the two types of females held virgin is compared with control mothers; but no obvious difference appears in this respect between curves

for the two types of virgin. It is relevant to obtain an estimate for this complete cycle, of the total retardation in egg deposition, for by the end of the time involved the eggs from early stored oocytes will have been laid. Discrepancies in deposition produced by the virginity are then almost exclusively attributable to retardation in egg production itself. The 4-day virgins gave for the four productive transfers when their recombination cycle ends at age 13 days, 529.3 adults per mother, corresponding to some 574.5 eggs laid per mother. Control mothers produced per mother to age 13 days (when their recombination cycle also ends) 799.4 adults, or 866.0 eggs. Thus the discrepancy amounts to some 34% of the control F_1 adults, or to 34% of the control eggs laid. If one considers the entire laying period of 12 days (to age 17 days for the 4-day virgins) the total yields, of course, are more nearly alike; however both the F_1 difference and the egg difference amounts to some 11%. The 4-day virgin is still somewhat behind the younger control mother.

For the 6-day virgin the comparable discrepancy in total egg yield for the recombination cycle (to age 15 days) is some 32% of the control yield (to age 13 days). But for the complete laying period of 12 days the 6-day virgin (at age 19 days) does overtake the control mother (at 13 days), for the former has produced a total yield of 872 eggs (as opposed to 866 for the control yield). The greater potential for egg production at the older age compensates here for the retardation in egg production suffered as a result of the prolonged virginity. (It is possible as previously noted that some disintegration of eggs has occurred here within the mother's body; however dissection by DR. JACK SCHULTZ of such females indicates, so he informs me, that this must be a relatively minor factor.)

The data of PATTERSON *et al.* (1932) showed a much greater F_1 yield per day from mated females than could be expected from the number of mature oocytes counted in the ovarioles of virgins plus the number of unfertilized eggs that virgins lay. They concluded: "The retention of mature eggs in the ovary greatly retards the development of the younger eggs." This implies that, after the formation of the earliest oocytes, and with sustained virginity, some feedback mechanism comes into play which retards oogenesis until mating occurs. As has been shown, results of the present paper support this hypothesis.

The initial effect of virginity is apparently to store oocytes and delay their deposition; thus by shifting the time at which recombination is measured, age effects on recombination undergo distortion. Through delay of egg production other influences come into operation which will independently affect recombination. If this latter delay had preceded chromosome exchange it would be expected, since for given eggs the mother is older than is normally the case, to provide an altered maternal environment for crossing over, and consequently to produce altered proportions of crossover gametes. However, judging by the early recombination values, it is most likely that the delay occurs after chromosome exchange. When mating takes place the retardation of egg production gradually disappears. These relationships will account for the larger number of transfers required by the control mothers to pass through the recombination cycle (taking six 2-day transfers instead of the four required by either type of virgin). There result both a longer period after the mating of the control mothers before their minimum

is reached; and a longer period after the minimum before their maximum is reached. If the virginity is continued a sufficient time other factors influencing recombination are introduced, as is evidenced by greatly increased egg mortality —these include the laying of unfertilized eggs, and the laying of presumably fertilized eggs which are so affected that they cannot complete embryonic development.

SUMMARY

Delayed mating of the mother affects variation with age of observed recombination values for the spindle fibre region of the third chromosome of *Drosophila melanogaster*. Significant differences are found between results from females mated immediately after emergence, females held virgin four days before mating, and females held virgin six days before mating. The general pattern of effects, during a 12-day period of egg laying after mating is similar for the three types of female in that the recombination value falls and rises in a regular manner with maternal age. However for control mothers, which were mated immediately, this cycle requires three 2-day transfers to reach its minimum value, as opposed to two such transfers after mating for either type of mother whose mating was delayed. Also three additional transfers are necessary for the high value to be attained by the control mothers, but only two transfers by either 4-day virgins or 6-day virgins. Thus both control mothers and 4-day virgins complete the cycle by the 13th day of age after emergence; the 6-day virgins complete it by the 15th day.—Measurements of the egg mortality, taken in conjunction with results of other workers on reproductive behavior in this form, indicate that the recombination effects shown by mothers whose mating is delayed depend upon: (1) storage of mature oocytes within the mother's body, (2) retardation of the development of younger germ cells, and (3) greater elimination by death among deposited eggs. In the present *st Sb*/+ females (1) and (2) must operate in all the mothers held virgin. For the mothers held virgin four days (3) is negligible; but for the mothers held virgin six days it has considerable weight.—The experiment has bearing on problems of experimental technique—also on problems arising in the interpretation of recombination data, especially if these data involve the use of agents which affect rates of egg production, egg laying, or egg mortality.

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