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The Effects of Low Temperature and Aging on Nondisjunction in Drosophila.[1]

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Footnote

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Footnote

1) For the purpose of this paper, the term "minimum food" stands for a food mixture which reduces the ability of females to ovoposit. A modified version of Burdick's medium (1954) was used. It consisted of 20 g agar, 10 g brewer's yeast, 43 g sugar, 4.3 sodium nitrate, 1.4 g dipotassium phosphate, 0.7 g potassium chloride, 0.014 g ferrous sulfate, 0.7 g magnesium sulfate, 0.14 g tegosept in 12 ml of 95% ethanol, and water added to make a final volume of 1000 ml (see Hildreth and Ulrichs, 1969).

INTRODUCTION

The genetics of nondisjunction of the X chromosome in Drosophila was first studied by Bridges (1913, 1914, 1916). He determined the frequencies of nondisjunction under standard conditions. Recently Hildreth and Ulrichs (1969) encountered variations in the frequency of nondisjunction when they subjected females to high (25°C) or low (10°C) temperatures during prolonged periods of aging preceding egg deposition. The experiments presented here were designed to obtain detailed data on the effects of temperature and aging on nondisjunction of the X chromosomes in females of Drosophila melanogaster, including data on successive broods of females treated in various ways.

MATERIALS AND METHODS

Nondisjunction was studied as evidenced among the F_1 of the cross y w ♀ X ++ (Samarkand wild-type) ♂. The y w P females were collected from stock cultures of the type y w ♀♀ X y w/ sc^8Y♂♂. The sc^8Y chromosome is a Y chromosome to which a small section of an X chromosome has been translocated. This section contains the dominant normal allele of y resulting in a not-yellow phenotype. Any XXY females in the stock would be recognizable by their not-yellow phenotype. Thus the use of y w females as P females in the experimental cross assured that all P females were XX.

In the main groups of experiments, the P females were newly eclosed virgins "pretreated" by being placed on "minimum food"[1] at 25°C for 3 days. After this common pretreatment period the females

were "treated" by being placed at 10°C or 25°C on minimum food for 0 day (control series "C-O"), or 3 days or 1, 2, or 3 weeks. During the aging periods of from 1 to 3 weeks the females were transferred from 1 to 3 times into new vials with minimum food. After the respective "treatments"-i.e., the temperature and aging periods exclusive of the 3 days of pretreatment--the females were placed on normal food (standard culture medium--corn meal, agar, molasses, yeast) and kept at 25°C. Each female was given the opportunity to mate with two wild-type males[2] (Samarkand), at least 3 days old, on the first and again the sixth and seventh day after treatment. Each treated female was kept in a separate culture vial and transferred to new vials daily until the fifth day, and then every second day until the 13th day. In the control C-O series, daily collections were made during the entire 13 days.

In the preceding paragraph the term "treatment" was defined as the period of aging under minimum food conditions that followed 3 days of pretreatment on minimum food at 25°C. Therefore the treatment periods of 0 or 3 days or 1, 2, or 3 weeks at 10°C or 25°C correspond to actual aging periods of 3, 6, 10, 17, or 24 days, respectively.

The number of eggs deposited in the treatment series during the first day after termination of treatment was less than in the succeeding broods. In order to obtain sufficient data on first-day broods the experiment with single P females was supplemented by mass matings using the same experimental procedures as in the main series except that only first-day broods were collected. To avoid overcrowding, a maximum of 15 females and 30 males per vial at 10°C and a minimum of 5

Footnote

1) In the following pages, the matings of single females with two males are somewhat inaccurately called "pair mating."

females and 10 males per vial at 25°C was used in each of these treatment series.

In addition to the control series C-O, in which the females were pretreated for 3 days and then placed on normal food, another control group, C, was reared which did not undergo pretreatment. This control is discussed later, as are also two special types of experimental series involved in the analysis of the first-day broods.

It is of importance for the interpretation of the results to know how many eggs are laid by virgin females during the pretreatment and treatment periods. During the pretreatment period 225 females of the C-O control series together laid 95 eggs, a mean of 0.42 egg per female (Table 1, line 1). For the experimental series, data are available for the number of eggs laid jointly over the pretreatment and early treatment periods or for later treatment periods alone (Table 1). For each period, the numbers of eggs laid per female were estimated by counting the approximate numbers of eggs in several randomly selected vials of the experimental series, each of which contained 30 females. As seen from the table, the estimated number of eggs deposited before mating is about five per female at the highest and less than one at the lowest among the various series. In other words, the loss of eggs in all series is very small. Granting this conclusion, the flies developing from the first broods represent germ cells which were mature eggs at the end of the treatment period and the flies developing in later broods represent increasingly earlier stages of germ cells at the end of treatment. In the normally developing ovariole, one can roughly estimate the duration of the period needed for the development of the various egg stages until

the time of their deposition as fertilized eggs (King 1957, Koch and King 1966). From such estimates, it appears that the first 3 days' broods are derived mostly from eggs that were present in the vitellarium as Stages 14 to 2 at the end of the treatment period, as well as from Stage 1 pro-oocytes in the germarium. The fourth- to sixth-day broods represent early Stage 1 pro-oocytes and clusters of 16-cell cystocytes, the seventh- to eight-day broods correspond to cystocyte division stages. Broods of the period 9–13 days after treatment represent cystoblasts and stem-line oogonia (Fig. 1).

Normal segregation of the X chromosome in the cross y w ♀ X ++ ♂ leads to wild-type females and y w males. Nondisjunction in the egg yields triple X, not-y, not-w females, y w females (XXY), and ++ males (XO). The triple X females have a very low viability, and hardly any develop to imagos. If they occurred at all in our cultures they were not distinguished from regular not-y not-w females. The y w females and ++ males were scored as products of nondisjunction in the female. Nondisjunction in the male alone does not usually lead to recognizable exceptional offspring, but coincidence of nondisjunction in both parents would lead to y w XX and ++ XY offspring. No attempt was made to distinguish between y w XXY and y w XX daughters, but ++ sons were tested for fertility: if fertile, they were XY, if sterile XO (presumably). Among the total 835 exceptional ++ males, 825 were scored as XO, which includes 18 ++ males that died before mating, and 2 ++ males that were added to the data by estimation (see footnote to Table 4). If nondisjunction of the XY pair in males is as frequent as nondisjunction of the X chromosomes in females, the expected frequency of ++ XY males would have been

1.5 (based on the data on males) instead of the observed 10. Thus, either all or some of the 10 fertile males were contaminants or the frequency of XY nondisjunction is several times as great as of XX nondisjunction. It seemed conservative to regard the 10 males as contaminants and not to include them in the data.

There were 10 ++ F_1 males which had an XO bobbed phenotype in regard to bristle length, and--often--abnormal chitinization and pigmentation of the abdomen. They had low viability and died without producing offspring. Most likely these males carried a mutant bobbed allele in their X chromosome, and their X sperm was of paternal origin and had fertilized a nondisjunctional O egg. The bobbed exceptions were scored as XO males, and included in the above total of 825 XO males.

Statistical analyses were based on the Chi-square test, using Yates' correction, when, given one degree of freedom, an expected class was less than five. The level of significance used is at the 1% probability unless noted otherwise. On account of the usually low frequency of nondisjunction, it was at times necessary to pool the data of some broods. The analysis of the data on nondisjunction is based on the female and male offspring jointly unless otherwise noted.

RESULTS

The main body of data on the frequencies of nondisjunction in the two control series and the eight experimental series is given in Tables 2 through 4. Numbers without parentheses include data on F_1 females

and males jointly. They constitute the material to be analyzed in the following pages. In addition, certain analyses are restricted to data on F_1 males only. These data are listed in Tables 2–4 within parentheses.

A. The effect on frequency of nondisjunction of aging P females at high temperature (25°C).

Each of the four experimental series shows a trend toward higher frequency of nondisjunction with increased aging treatment (Table 3, last line).

A comparison of the totals of the four experimental groups with that of the control C-O shows an increase of nondisjunction in each experimental group, the differences being significant at the 0.2% or still lower level (Table 5, line 1).

These overall effects can be analyzed in terms of the nine brood collections, which correspond to stages from mature eggs to stem-line oogonia at the end of the treatment period.

In each experimental series, the frequency of nondisjunction is higher in the later than in the earlier broods (Table 3, Fig. 2). The border line for this phenomenon seems to be represented by the fifth-day brood. There are three apparent exceptions to this age effect. These are the first-day broods of the 1-, 2-, and 3-week treatment series, which give higher nondisjunction frequencies than the next three broods. Statistical analysis shows, however, that the relatively high frequencies in the first-day broods of these series are not significantly different from the sum of the second- to fourth-day broods of the same series (Table 6, line 4), and the first-day brood of the control C-O does not differ significantly from the sum of the first-day broods of the three experimental

series ($\chi^2 = 0.30$, d.f. = 1, P = 0.5–0.7).

On the other hand, the pooled data of the 1-, 2-, and 3-week series show a significant difference in frequency of nondisjunction between the sum of the first broods and the sum of the second- to fourth-day broods (P = 0.01). A similar comparison involving pooled data from the 3-day and 1-, 2-, and 3-week series are significant at the 7–8 % level only, and a comparison involving pooled data of the control C-O and the four experimental series yields significance at the 5–7 % level.

As is shown later, the second- to fourth-day broods in each experimental series at 25°C do not show any effect of aging. In contrast to this the preceding analysis indicating that the first-day brood aged at 25°C shows a tendency to increased frequency of nondisjunction with increased period of aging, from 1 to 2 or 3 weeks. This tendency, if real, is of minor nature, and more data are required to establish its reality.

The differences between the early and late broods were analyzed further by subdividing the broods into two subgroups, comparing the first- to fourth-day broods with the fifth to the last (5–13th day) broods. Chi-square tests between the two subgroups show no difference in the control C-O (P = 0.3–0.5), a difference significant at the 3% level in the 3-day treated series, and differences significant below the 0.1% level within the 1-, 2-, and 3-week treated groups (Table 6, line 1). When the two subgroups were composed of the first- to fifth-day and the sixth- to last-day broods, very similar results were obtained (line 2). One may conclude that there is a significant difference between the early and late broods of all four experimental series but not in the control C-O.

Further tests relate to the question whether there are differences between the early and late subgroups of the control vs experimental series. No significant differences were found between the first- to fourth-day broods (Table 5, line 2), whereas the fifth- to last-day broods differed at less than 0.1% probability (Table 5, line 3). Homogeneity tests for the early subgroups showed nonsignificant differences ($\chi^2 = 2.40$, d.f. = 4, $P \doteq 0.5$—0.7), whereas tests of the late subgroups showed highly significant nonhomogeneity ($\chi^2 = 200.7$, d.f. = 4, $P < 0.001$).

Additional comparisons between different early broods within the control and experimental series showed no aging effect during the first- to fourth-day brood period. On account of the small numbers of nondisjunction individuals in these broods it was necessary to group the four broods in sets of two: first- plus second-day broods vs third- plus fourth-day broods. All comparisons of the two groups show statistical homogeneity (Table 6, line 3). In addition, comparisons between the sums of the second- to fourth-day broods for the control C-O and for the four experimental series also show homogeneity (Table 5, line 6), in contrast to the highly significant differences between the frequencies of nondisjunction in the fifth- to thirteenth-day broods of the control C-O and the various aged series (line 3).

In order to analyze the aging effect on nondisjunction between the individual fifth- to thirteenth-day broods, each brood of the various experimental series was compared with the corresponding group of the control C-O series. Since the two 10—11th- and 12—13th-day broods represented somewhat small samples, the data were combined and the 10—13th-day broods were treated as a single group. The only significant difference between the control C-O and the 3-day-treated series was that between

the 6—7th-day broods (Table 5, lines 7-10, column 3). Between the 1-week-treated series and the control there was a significant difference not only relating to the 6—7th-day broods but also relating to the 8—9th-day and 10—13th-day broods; the only not significant difference related to the 5th-day brood (column 4). Comparisons between the control C-O and the longer-treated 2- and 3-weeks series showed significant differences for all brood groups (last two columns). It thus appears that the 6—7th-day brood is most sensitive to the aging effect at 25°C under minimum food condition. The 8—13th-day broods are next in sensitivity, with the 5th-day brood being least sensitive.

In summary, aging at 25°C increases the frequency of nondisjunction in the later broods. The 6—7th-day brood is the most sensitive to aging, the 8—13th-day broods are next, and the least sensitive 5th-day brood also can be affected by longer periods of aging. In the earlier broods there is no effect of aging except for the first brood in pooled data of the 1-, 2-, and 3-week treatment series, which give significantly higher frequencies of nondisjunction in first-day than in the 2—4th-day broods, ($P = 0.01$).

B. The effect of aging P females at low temperature (10°C)

1. Comparisons with C-O controls

Table 4 and Figure 3 provide the data for the various series of P females aged at 10°C. On the whole, the pattern of effects of aging in increasing the frequency of nondisjunction is similar at 10°C and 25°C. There is, however, a striking difference between the effects of the two temperature treatments on nondisjunction in the first broods.

Comparisons between the 25°C control C-O and the various low-temperature series show significant increases in nondisjunction of the sums of all broods in all four treated series (Table 7, line 1). The same is true for the sum of all broods except the first (line 2), and for the sums of the 5—13th-day broods (line 4). For sums of the 2—4th-day broods, only the 3-week treated series differs significantly from the control C-O (line 3).

Broodwise comparisons between treated and control C-O series show no significant differences for 2nd-, 3rd-, and 4th-day broods and the 3-day and 1-week treated 5th-day broods (Table 7, lines 5-12). The exception is the 3rd-day brood of the 3-week treatment series, discussed later. After treatments of 2 and 3 weeks but not for shorter periods the 5th-day broods have significantly higher nondisjunction rates than the control C-O (line 9). The same holds true for most of the comparisons for 6—7th-, 8—9th-, and 10—13th-day broods (lines 10-12), with only borderline significance reached by the 3-day treated series for the last two brood groups. The lack of effect of treatment on the early broods and the strong effects on the 6—7th-day broods correspond to the findings from high-temperature treatments, whereas there is a contrast between the two temperature effects for the later broods.

The greatest difference concerns the first-day broods. In contrast to the 25°C series, for which there was no significant difference between first-day treated and control C-O broods, all 10°C treated series--3 days, 1, 2, or 3 weeks--produce first-day broods with very highly increased frequencies of nondisjunction (Table 4, line 1; Table 7, line 5). The first-day broods are analyzed below in detail.

2. Comparisons with high-temperature aged series

In the preceding section the frequency of nondisjunction in low-temperature-aged females was compared with that in high-temperature control C-O. The treated series thus is different from the controls in two attributes, temperature and age. In order to separate the influences of these factors, the results of the various low-temperature-aged series were compared with those of the high-temperature series of equally aged females. (It is likely that the general physiological effects of aging at the two temperatures are not identical. However, no adjustment for such differences have been made. On the whole this did not seem necessary.)

Comparisons between the totals of all 13th-day broods at high and low temperatures show no significant difference in the 3-day aged series but highly significant differences in the 1- and 2-week series, and a difference at the 5–6% level for the 3-week series (Table 8, line 1). These significant differences are primarily due to the exceedingly high frequency of nondisjunction in the first-day broods of the low-temperature series. This is shown by comparisons between the first-day brood of low-temperature-treated series and the sum of all others (Table 6, line 7), and between the first-day brood and the sum of the 2–4th-day broods (line 6). Similarly, the first-day brood of all low-temperature series differs significantly from those of the corresponding high-temperature series (Table 8, line 4). These differences in the first-day broods between the high- and low-temperature series show that it is the temperature and not the aging treatment that is responsible for the observed differences.

In order to test for differences between the high- and low-temperature series independently of the first-day broods, the sum of the 2—13th-day broods was used for comparison (Table 8, line 2). Three of the four comparisons yielded non significant differences and the fourth, relating to the 2-week treatment, was significant at about the 2% level. Thus, there seems to be only a minor temperature effect, if any, for the sum of 2—13th-day broods.

Further analysis showed some minor temperature effects on certain broods of specific aging series. One of these concerns the third-day brood, which does not show a temperature effect in any but the 3-week aging series (Table 8, line 6). This is similar to the situation in which the sum of the 2—4th-day broods are compared with the control C-O (Table 7, line 3). Three of the latter comparisons show no significant differences, but the fourth, involving the 3-week series, does reach significance. Specifically, the difference between the 2—4th-day broods of the high- and low-temperature 3-week series may be assigned to the 3rd-day brood, but only at a 3% level of significance (Table 8, line 6).

Table 8 includes also data on the later broods of the low- and high-temperature series. The significant difference found earlier for the 2—13th-day broods of the 2-week treated series seems to be dependent on a difference in the 6—7th-day broods. Two of the four comparisons yield nonsignificant differences but the two comparisons remaining, those for the 1- and 2-week treated series, are significant at about the 2% level (line 9).

In summary, low-temperature treatment profoundly increases the frequency of nondisjunction in the first-day brood. Later broods are mostly not, or in two cases only slightly affected. One of these, the 3rd-day brood, is affected only by the longest treatment period (3 weeks), the other, the 6–7th-day brood, shows an effect after 1- and 2-week treatment.

C. The effect of aging P females as shown by their male offspring

The foregoing analyses were based on the total offspring of treated and control P females. It seemed desirable to repeat the analysis using the male offspring only. This restriction to the male sex has two advantages. For one, it has long been known that the frequencies of female and male nondisjunctional exceptions are unequal, the male exceptions being much more frequent than the female exceptions. This preponderance of male exceptions has been attributed to a process in which the two X chromosomes do not disjoin normally, nor move together in typical nondisjunctional fashion. Instead, it is assumed that a single X chromosome gets included in either the egg or first polar nucleus whereas the other X chromosome lags on the meiotic spindle and does not get included in either nucleus. Fusion of sperm and X-bearing egg nucleus results in regular female and male offspring, but fusion of sperm with a no-X-bearing egg nucleus results in inviable OY and exceptional male F_1 (Morgan, Bridges, and Sturtevant 1925). This is borne out by these experiments, including control C-O and eight series of high- and low-temperature treatment, which yielded 318 female and 667 male exceptions. It is noteworthy that the hypothesis of lagging of an X

chromosome does not seem to apply to all broods of the C-O and high- and low-temperature series. The first-day broods of the low-temperature series seem to show a rather substantial tendency toward equality of the sex ratio of exceptions seen in the last column of Table 4, line 1. Here the ratio is 58 ♀♀ : 72 ♂♂, P = 0.1–0.2 for deviation from equality. The clarification of this interesting problem will depend on future study.

A second advantage of using the data on males only consists in the greater certainty that exceptions are of nondisjunctional origin and not due to contamination. These two possibilities cannot be distinguished among female exceptions, but male exceptions, if actually due to contamination, can be distinguished from nondisjunctional XO exceptions by their fertility. As stated in the Material and Methods section, only 10 fertile exceptional males were found. They either were contaminants or originated as the results of simultaneous nondisjunction in both parents. They were excluded from the analyses, but this exclusion does not affect the data to any noticeable degree. There is, of course, one important disadvantage to the restriction of the analyses to data based on males only. This is the reduction of the number of individuals available for analysis.

The same types of analysis were performed on the male data as those shown in Tables 5 through 8 for the total data. The results were similar, and almost exactly the same conclusions can be derived. Only minor differences that might affect the interpretations were encountered. Those are listed as footnotes in Tables 5 through 8. Most of the differences can be understood as due to insufficient sample sizes, especially in the cases that are borderline as to effectiveness of treatment. Among them

the following deserve special mention.

1. The effect of low temperature on the first-day female and male brood of each experimental series was found again in the males-only data except in the case of the 3-day treatment, as shown in Table 7, line 5, and Table 8, line 4. This seems to indicate that the 3-day treatment period is close to the borderline of the length of effective low-temperature treatment. The absence of a first-day brood effect among the male offspring of the 3-day series and also the earlier discussed patterns of the effect on both sexes according to the length of treatment are important for the interpretation of the influence of temperature. They show that the effect of low temperature on the first-day brood is not simply caused by the shock of changing the P females first from 25°C to 10°C and then from 10°C to 25°C.

2. When the 1-week low-temperature series is compared with control C-O, the 2—4th-day brood differs from the first-day brood at a 0.06—0.08 probability level in the joint female and male data, whereas the males-only data show a 0.02-0.03 probability (Table 7, line 3, column 4). The 4th-day brood alone shows a 30—40% probability in the joint female and male data, whereas the males-only data yield the difference at the 4—5% level (line 8, column 4). Since none of the high-temperature series nor the 3-day and 2-week low-temperature treatment series show any significant difference between the 2—4th-day broods and the C-O series, this is interesting to note.

3. A low-temperature treatment of 3 weeks produced an increase in nondisjunction in the third-day brood over that at high temperature with a probability of 3% (Table 8, line 6, last column). The male data

give a similar result, with a 2% probability. Actually, the frequency of nondisjunction in this low-temperature group happens to be determined solely by the frequency of exceptional males (20), there being no exceptional females. The effect is not drastic, but it suggests that the very long period of 3-week low-temperature treatment exerts an influence toward increase in the frequency of nondisjunction.

D. Analysis of the first-day broods

The first-day brood in each of the low-temperature-treated series shows a greatly increased nondisjunction frequency in comparison with the control C-O and the high-temperature series. For the 3-day low-temperature series, the frequency of nondisjunction for females and males jointly in the first brood is nearly 0.6%, and for the 1-, 2-, and 3-week treated series it is 5.14, 8.11, and 5.55% respectively. These frequencies are from 10 to 140 times those of the first-day broods of the control C-O and from 17 to 65 times those of the corresponding high-temperature-treated series. The following analysis is intended to determine the stage in oogenesis that is responsible for the unusually high frequencies of nondisjunction.

Each of the first-day broods came from eggs collected within 24 hours after the treated females were given the opportunity to mate. During this period only few eggs were laid. This accounts for the rather small number of offspring obtained in each case in comparison with subsequent egg collections. The actual sizes of first-day-brood sibships from pair-mated parents, control C-O, or treated with high or low temperatures, are given in Table 9 together with the frequencies of non-disjunctional exceptions among them. As seen from the table, the

majority of treated females produced only one or two offspring during the first day of egg collection. The highest number was 25, from 3-day treated females. The highest number of siblings among which nondisjunctional flies appeared was 10 (two cases from two P females), from 3-day treatment, low temperature. All other nondisjunctional exceptions came from sibships of 8 or less. These facts strongly suggest that the nondisjunctional offspring of the first-day broods came from the first 10 fertilized and viable eggs deposited by a treated P female after the first mating.

Under normal conditions the number of functional ovarioles in Drosophila melanogaster has been estimated as between 10 and 30 per ovary (King 1957, Koch and King 1966). If the most mature oocyte at the distal end of each ovariole is inseminated and develops successfully before the rest of the oocytes, one can expect 20 to 60 offspring per female from eggs that were mature at the time of the first mating. The fact that the first-day brood from the low-temperature-treated female shows the striking effect on nondisjunction frequency only among sibships of less than 10 leads to the conclusion that the effect is on the mature eggs. This is also true when the number of 10 mature eggs is increased by 5, the latter being the highest estimated number deposited during the whole treatment period (Table 1).

This conclusion is supported by the outcome of a special experiment in which newly eclosed females, instead of being pretreated at 25°C for 3 days, were placed immediately on minimum food at 10°C for 3 days and then transferred to normal food at 25°C and given the opportunity to mate. (All subsequent handling, including brood collections,

were the same as for the control C-O.) In the newly eclosed females the most advanced eggs are in Stage 7. Under normal conditions it takes at least 5 hours to proceed from the beginning of Stage 8 to Stage 14. It then takes an estimated 9.58 hours in Stage 14 to reach full maturity (King 1957). Thus at least 15 hours are required by newly eclosed females to produce mature eggs ready to be fertilized. If the first-day-brood effect of low-temperature-treated P females on nondisjunction is exerted on mature eggs, no increase of nondisjunction over other than low-temperature-treated flies was to be expected from the not-pretreated P females. The first-day brood collection from 420 P females (120 pair mated, 300 mass mated), yielded only a single normal segregant male. The second-day brood consisted of a total of 9 493, including four nondisjunctional males (0.04%). Based on males only, four out of 4 741 were nondisjunctional (0.08%). This second-day brood, obviously, is not derived from treated mature eggs, but from earlier stages, which do not show a low-temperature effect although they had been exposed to 10°C for 3 days. A χ^2 test applied to the sum of the first two broods from nonpretreated 3-day low-temperature-treated females compared with the first-day brood of the control C-O showed complete homogeneity ($\chi^2 = 0$, $P = 1$; the same result with males only) but a comparison of the first two broods from nonpretreated 3-day low-temperature-treated females with the first-day brood of the pretreated 3-day low-temperature-exposed series yielded significant differences ($\chi^2 = 29$, $P < 0.001$; males only $\chi^2 = 6.08$, $P = 0.01$–0.02).

The data presented in Table 9 show a tendency to decreased size of sibship with increased period of aging in both high and low temperatures. This phenomenon may be due to the presence of disintegrated or

unfertilized mature eggs as a result of the treatment, a situation reported by Patterson et al. (1932) in their studies of the joint effect of aging and of X-radiation on the frequency of nondisjunction. It would mean that the longer the treatment period the more abnormal eggs will be produced. An alternative hypothesis is that the treated females require some time to recover from the treatment, that this recovery delays egg laying, and that the recovery period is positively correlated with length of treatment.

If the second hypothesis is valid, one would expect an increased frequency in nondisjunction in the second brood from at least the low-temperature females treated for the longest time. As shown earlier, no such effect is found in the second-day broods of any treated series. A similar consequence of the second hypothesis relates to the first fertile brood collection made on the second day after mating of those females who did not produce viable offspring during the first day. If the second hypothesis is correct, one would expect an increased frequency of non-disjunction in these selected second-day broods. This, however, is not the case, as shown in Table 10. A direct check on the second hypothesis would be obtained by counting of eggs deposited during the first-day period that had not developed to adults. Such data are not available for these experiments, but closely related data are available for the 2-week low-temperature-treatment series. Here, those vials intended for first-day brood egg collections which failed to produce any viable offspring were checked for the presence of eggs laid. From 165 pair matings, a total of 17 vials did not produce offspring but contained a maximum estimated number of 10 eggs per female. In 11 of the 17 vials, second-day brood collections produced a total of 236 offspring, none of which

were the results of nondisjunction. These findings support the first hypothesis by showing that there are eggs among the first-day brood collection that fail to develop and that there is no low-temperature effect on the second-day collection even if this is the first fertile brood.

If, as suggested, the effect of low-temperature treatment is on mature eggs, one must conclude that the second-day broods are not derived from mature eggs present at the end of the low-temperature treatment. Otherwise these broods should show high nondisjunction rates. The same reasoning applies to each of the main experimental series in which the first-day brood collections but not the second included mature eggs present at the end of the temperature treatment. In these considerations, it is implied that the low-temperature effect extends over at least 24 hours after removal of the females from treatment, since production of the second-day brood begins only 24 hours after removal. If it extended over a shorter period, one would not expect an increased nondisjunction frequency in the second-day brood.

This point was tested by a special experiment. It consisted of the following procedures. The low-temperature experiments of 1 and 2 weeks' treatment time were repeated in mass matings and, at the end of treatment the P females were transferred to minimum food at 25°C for 24 hours. They were then placed on normal food and mated, and first-day broods were collected. These broods did show increased nondisjunction frequencies. During the 24 hours at 25°C before mating, a total of 550 P females which were treated for 1 week at 10°C deposited 12 eggs (= 0.02 egg per female), and a total of 1350 P females treated for 2 weeks at 10°C deposited 164 eggs (= 0.12 egg per female). In the

1-week-treated series nondisjunction occurred in 3.27% (62 exceptions out of 1892 flies; male data only: 29/ 927), and for the 2-week-treated series, in 6.66% (135 out of 2 027 flies; male data only: 67/1024). Compared with the 1-week low-temperature series (Table 4, line 1), which gave 5.13% of nondisjunction, the 3.27 percentage is significantly different, while the 6.66 percentage is not significantly different from 8.11% of the 2-week low-temperature series (line 1). However, the two percentages, 3.27 and 6.66, are so much higher than all but first-day brood frequencies that there is no doubt that the low-temperature effect persists more than 24 hours.

In order to obtain information on the state of the ovaries, egg counts in the ovarioles of the P females were made immediately after the 2-week high- and low-temperature treatment. The ovaries were dissected out, fixed, stained by the Feulgen method, and mounted on a slide, and the number of Stage 14 eggs was determined under the microscope (following the method used by King, Burnett, and Staley 1957). Some of the P females had an egg in the uterus that was excluded from the count.

In each series, 25 P females (50 ovaries) were studied. The females with low-temperature treatment showed an average of 7.36 ± 0.47 (standard error), and a range of 4 to 12 eggs at Stage 14 per female (1 to 7 per ovary). The females with high-temperature treatment showed an average of 67.56 ± 2.69, and a range of 37 to 88 eggs per female (14 to 53 per ovary).

These data compared with the number of F_1 from the P females treated in the above manner and shown in Table 9, columns 4 indicate

the following. In the low-temperature series, the highest number of F_1 per female was 10 (average 1.4) and the highest number of mature eggs per female was 12 (average 7.3), whereas in the high-temperature series, 17 (average 2.6) and 88 (average 67.5) were the comparable numbers, respectively. There were thus more mature eggs present in the ovaries than corresponding numbers of F_1 individuals of the first-day brood.

It had been suggested earlier that the difference was due to nonfertilization or disintegration of eggs. While counting the mature eggs, however, severe disintegration was not observed. Only occasional accumulation of a small amount of black pigment in the egg, and in very rare cases abnormal appearance of the cytoplasm, was noticed. In the low-temperature treated ovary, where few mature eggs were observed, 10 or more ovarioles were present in most of the cases. Most of them contained only earlier stages of the ova. It seems likely that the oocyte, rather than having disintegrated, did not noticeably develop. This assumption is well supported by the study of ovaries at the end of the 3 days pretreatment on minimum food at 25°C. From the 25 females (50 ovaries), the average of 6.0 ± 0.52, and a range of 3 to 12 eggs at Stage 14 per female (0 to 7 per ovary), was observed. During the following 2-week low-temperature treatment, these Stage 14 eggs are retained in the ovary and consequently show an extremely high nondisjunction frequency at meiosis. In the high-temperature series, the presence of the large number of eggs is apparently due to the presence of retained eggs. This will be discussed later.

E. Analysis of control series

The foregoing results of the effect of aging on nondisjunction frequency in the experimental series involved P females who had been kept on minimum food. In each series, aging effects were apparent in the later halves of the broods, which include eggs that during treatment had been in the whole range of oogonial to cystocyte stages (Fig. 1).

This section deals with tests made with females cultured under normal conditions.

Newly eclosed females were placed at 25°C with males (at least 3 days old) on normal food. Daily egg collections were made from the day of eclosion until the 16th day. During this period P males were present from the first to the fourth day and again during the ninth and tenth days. In this control "C" series, 120 pair matings were used, supplemented for the first 4 days by mass matings involving 300 females.

The frequencies of nondisjunction varied greatly, probably largely due to the very small percentages (Table 2). More data will be required for a fully valid analysis. It is, however, already possible to draw certain conclusions if one groups broods in a manner used in earlier discussions. If one divides the C series into four successive groups the following facts appear. The first group, consisting of broods 1–4, shows 0.029% nondisjunction (15/50 153); the second group, consisting of broods 5–8, shows 0.071% (13/18 214); the third group, consisting of broods 9–12, shows 0.075% (13/17 209); and the last group, consisting of broods 13–16, shows 0.101% (11/10 839). A χ^2 test shows the four group to be heterogeneous: $\chi^2 = 12.33$, d.f. = 3, P = 0.007, suggesting that the later broods tend to have an increased frequency of nondisjunction. There is also heterogeneity when the broods are divided into 2 groups only, 1–8 vs 9–16: $\chi^2 = 7.39$, d.f. = 1, P = 0.007.

As seen earlier, the first- to fourth-day broods of the various experimental series at high-temperature treatment as well as the control C-O were very similar despite their differences in aging treatment. In the control C series, the corresponding broods are those of the first to the fifth days as judged by the very small number of eggs laid on the first

day. This small number is accounted for by the procedure which led to egg collection immediately after eclosion, whereas a minimum of 15 hours is required to develop eggs ready for insemination and deposition. For the same reason the 13th day of the experimental and C-O series is represented by the 14th day of the C series. If one tests for homogeneity between the 1–5th-day vs the 6th–14th-days grouped broods, one finds a significant increase of nondisjunction frequency in the latter groups: $\chi^2 = 17.17$, d.f. = 1, $P < 0.001$. If a test is made using the P female's age as a dividing criterion instead of the brood number, one must consider that the females of the C-O series went through 3 days' pretreatment, so that the first- to fourth-days broods of the C-O controls come from females corresponding to the fourth to seventh days age of the not-pretreated C controls. With these ages used as dividing markers, a test of the 4–7th-day vs the 8–16th-day broods of the C series also shows a significant difference: $\chi^2 = 6.29$, d.f. = 1, P = 0.01-0.02. In the C-O series, on the contrary, a comparison between the 1–4th-day vs 5–13th-day grouped broods does not yield a significant difference (Table 6, line 1). Such differences do appear, however, when the C-O broods are grouped in smaller units, e.g., 1–4th-, 5–9th-, and 10–13th-day broods or 1–3rd-, 4–5th-, 6–7th-, 8–9th-, and 10–13th-day broods: for the comparisons involving three groups, $\chi^2 = 7.70$, d.f. = 2, P = 0.02–0.03; for the comparisons involving five groups, $\chi^2 = 12.72$, d.f. = 4, P = 0.01–0.02. Similarly, when the early 1–4th-day broods of the C-O series are compared with the last (10–13th-day) broods, the latter is higher in nondisjunction frequency than the former at a 2–4% significance level. Corresponding comparisons in the C series give

highly significant differences: 1–4th-day group vs 11–14th-day group, $\chi^2 = 6.93$, d.f. = 1, $P = 0.009$; 1–5th-day group vs 13–16th-day group, $\chi^2 = 12.32$, d.f. = 1, $P < 0.001$.

These data permit the conclusion of that both C-O controls with 3 days' aging pretreatment and the C controls without such pretreatment show a tendency to increase the nondisjunction frequency in the later part of the life of the P females during the 13 or 16 days, respectively, of their egg laying. Whether the relative difference between the two control series in the intensity of the aging effect is caused by the difference in pretreatment of the two control series (3 days vs none) or is due to statistical variability remains unknown.

F. Absence of an effect of mating on nondisjunction frequency

In each experimental series, effects on nondisjunction were apparent in 6–7th-day broods, and, at low temperature, such an effect was seen in the first-day brood. It happened that the first and 6–7th days were those on which the females were given the opportunity to mate. This association between increased frequency of nondisjunction and mating suggested the possibility of a causal relation. This possibility can be ruled out. First, in the C-O controls, neither the first- nor the 6–7th-day broods show any significant increase of nondisjunction over the remainder. Second, in the C controls, for which males were available for the first 4 days and 9–10th day also no significant increase in nondisjunction occurred. Third, in the experimental series none of the first-day broods of the 25°C experiments show a significant increase in nondisjunction. For the 5th-day broods a trend toward an increase of

nondisjunction is observed which is not statistically significant for the 1-week treatment series (or less), but which reaches significance for the longer treatment series. The fact that it is also significant in all experimental series for the 6–7th-day brood is apparently independent of the mating event.

DISCUSSIONS.

Evidence for an association between maternal age and nondisjunction of chromosomes in humans has long been known, particularly for chromosome 21 (see Penrose and Smith 1966) and the sex chromosome (Lenz, Nowakowski, Prader, and Schirren 1959). In Drosophila, no proof for such an association has been furnished (see Kersall 1963) except when aging is combined with X-ray treatment (Patterson, Brewster, and Winchester 1932, Uchida 1962). The latter combination is also effective in humans (Uchida, Holunga, and Lawler 1968).

This paper reports on aging effects at 25°C on both early and late stages of oogenesis in Drosophila, from oogonia to early cystocytes. These effects become evident as nondisjunctional events in meiosis. The most sensitive stages are represented by the 6–7th-day broods. It is estimated that these broods correspond to the period of cystocyte division and early cystocyte 16-cell clusters present during aging (Fig. 1).

It is at the end of this period, about 6 hours after completion of the 16-cell cluster stage, that the synaptonemal complex first appears (Koch, Smith, and King 1967). This complex in general is not regarded as either responsible for, or necessarily a consequence of, pairing,

though chromosome conjugation must precede its formation (Moses 1969). Yet it is suggestive that the high sensitivity for aging effects of the ovarian stages which correspond to the 6–7th-day brood is in some way related to the beginning of formation of the synaptonemal complex, or to the preceding chromosome pairing, which might lead to faulty synapsis later expressed as nondisjunction in the mature egg. This interpretation follows the reasoning of Smith and King (1968), who studied the gene c(3)G in Drosophila which fails to permit formation of the synaptonemal complex and results in abnormal disjunction. These authors suggested that "a major function of each synatonemal complex is to insure proper meiotic disjunction of the component chromosomes in a bivalent, whether or not crossing over has occurred between them."

The aging effect in the experiments reported herein is not exclusively brought about by way of the synaptonemal complex. This follows from the fact that for the longer periods of aging treatment the earlier cystoblast cells and the stem-line oogonia are also affected, although these stages precede formation of the synaptonemal complex. In addition, no aging effect was observed among the first- to at least the fourth-day broods which correspond to the mature egg Stage 14 and late cystocyte stages present at the end of the treatment period. The synaptonemal complex is still in formation during part of this period. The absence of an aging effect in spite of the presence of the complex shows that aging and the later stages of the formation of the complex are not interconnected.

The aging effect on nondisjunction occurred not only in the experimental series with storage on minimum food, but also in untreated control females. In the C control, where daily egg collections under

normal conditions were carried out for 16 days, there was a clear trend toward increasing frequency of nondisjunction in the later broods. Within this control series as within the experimental series the effects of aging on different ovarian stages are compared: earlier broods reflect the pro-oocytes and consecutive stages up to mature eggs, later broods oogonia, cystoblasts, and cystocyte stages.

Obviously, these different sequences are not fully comparable, when the effect of the mother's age on nondisjunction frequency is considered. It will be desirable to extend study of the age effect in normal development by comparing the frequency of nondisjunction in females whose age differences include the whole cycle from oogonia through mature eggs. At maximum efficiency this cycle takes at least 9 days (Fig. 1). Comparisons should therefore be made either between an early 10-day-broods period and a later 10-day period or by disregarding the first 10-day period and comparing broods within the second or between later periods.

The drastic low-temperature effect found in the first-day brood in the experimental series requires a minimum duration of the treatment period. It is not the result of the temperature shock experienced upon the initial change from 25°C to 10°C and the terminal change back to 25°C. The drastic low-temperature effect is restricted to mature eggs, and the effect is maintained at least 24 hours after cessation of the low-temperature treatment.

The slight effect of low temperature on the 6–7th-day broods as seen in the 1- and 2-week-treated series, when compared with the effect of the high-temperature series on the same broods, suggests that the

aging effect was enhanced by its combination with low temperature. This increased effect was, however, not found in the data on males only, nor in the 3-week low-temperature treated series. Only more data can show whether the effect is truly present.

Another effect on nondisjunction demands further work. It is the effect in the 3-week low-temperature series on the 3rd-day brood. This brood, in normal development, corresponds to the Stage 1 pro-oocyte at the end of the germarium to the early pro-oocyte in the vitellarium. The unique 3-week effect may have been involved in the pioneering experiments of Hildreth and Ulrichs (1969), in which the low-temperature treatment was also 3 weeks. Their data, which were not directly divided into broods, covered offspring corresponding to the first-, second-, and in part the third-day brood. Thus the effect on nondisjunction observed by them must have been caused mainly by the effect on the first-day brood and, to a minor degree, on the third-day brood (there seems to be no effect on second-day broods).

Studies on amphibians and nonhuman mammals have led to various assumptions regarding the cause of nondisjunction. One of these assumptions suggests postovulatory overripeness of normally mature primary oocytes (Witschi and Laguens 1963, Butcher and Fugo 1967, Mikamo 1968). Based on studies on Xenopus, Mikamo hypothesizes that nondisjunction is due to degeneration of spindle fibers induced by intrafollicular overripeness of the egg (1968).

In humans, it has usually been assumed that aging effects the eggs prior to ovulation, but German (1968) has suggested that at least part of the aging effect is exerted after ovulation.

In D. melanogaster, the eggs involved in producing the first-day brood in the present experimental series of high- and low-temperature treatment correspond to eggs exposed to overripeness as in the studies just cited. The weak effects of aging apparent in later broods are based on influences exerted on younger egg stages. These aging effects correspond to those in the majority of human cases of nondisjunction of chromosome 21 induced in immature egg stages.

The high-temperature series did not show a clear-cut effect on nondisjunction in the first-day brood, although a tendency toward increasing nondisjunction with increasing length of aging existed. More data are needed to substantiate the possible effect of overripeness independent of temperature in Drosophila, but the effect, if any, will be very minor in comparison with the effect by the combination of aging and low temperature. It is a possibility that the cause of the high nondisjunction frequency in the latter case is the consequence of the abnormal spindle formation at the meiotic divisions. Beside the Xenopus case, abnormalities of the spindle involved in nondisjunction have been observed in eggs from homozygotes for the "claret" nondisjunction gene in Drosophila simulans (Wald 1936). In analogy to the situation in D. simulans, Davis (1969) suggested that nondisjunction in a homozygote for the "claret nondisjunctional" gene in D. melanogaster is also due to abnormal spindle formation. No cytological observations were made in this case.

One of the interesting findings of the present study is that the responses of the female germ cells in various developmental stages to different factors such as aging and temperature ultimately result in

the same phenomenon: increased frequency of nondisjunction at meiosis. The suspected relations between aging and the mechanism of chromosome pairing, and between low temperature and meiotic spindle formation, require future studies. It is hoped that the drastic low-temperature effect on the first-day brood will become a useful tool for clarification of the nature of the process of nondisjunction.

SUMMARY

Females of Drosophila melanogaster were subjected during a premating treatment on minimum food to various periods of aging from 3 days up to 3 weeks at 25°C and 10°C. The effects of these treatments were measured as increases of the frequency of nondisjunction of the X chromosomes.

No aging effect at 25°C was found among the first- to at least fourth-day broods, which represent germ cells from mature eggs to a late cystocyte stage in the treated females, although there was a possibility of the presence of a minor effect on the first-day brood when aging was longer than one week. The effects were found in later broods corresponding to cystocytes up to stem-line oogonia at the end of treatment. The most sensitive stage is that of the 6–7th-day broods, as shown by aging series of 6 days (including 3 days' pretreatment period) and longer. A relationship is suggested between the aging effect on nondisjunction and the early stages of formation of the synaptonemal complex.

An age effect in the P females on nondisjunction is also apparent in flies raised under continuously normal conditions. In a study of 16

successive daily broods, the later broods had increased frequencies of nondisjunction as compared to earlier broods.

An exceedingly strong increase of nondisjunction occurs in the first-day broods after 1 or more weeks of treatment of the P females at low temperature (10°C). The frequency of nondisjunction is as high as 140 times that found in controls and experimental series not involving low temperature. Even low-temperature treatment of only 3 days' duration is at the border line of effectiveness. It is not the temperature shock involved, but a certain minimal length of exposure to low temperature that is responsible for the striking increase of nondisjunction. The effect is almost exclusively based on influences on the mature eggs, and extends over at least 24 hours after removal from treatment. It is suggested that the low-temperature treatment may interfere with spindle formation and thus lead to nondisjunction. A weak low-temperature effect has also been observed in the 6–7th-day broods of the 1- and 2-week-treated series as well as a weak effect on the third-day brood of 3-week treated females.

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Legend for Figures.

Figure 1. Schematic representation of the estimated timing of egg development in an ovariole operating at maximum efficiency (after the studies of King 1957, Koch and King 1966, and Koch, Smith and King 1967). The intervals on the time scale are not proportional to one another. Also, the dimensions of the different stages are not proportional to one another. (For instance, the maximum lengths in μ for the chamber stages 1, 2, 4, 6, and 14 are 15, 20, 40, 65, and 450, respectively.)

Figure 2. The frequencies of nondisjunction in each brood of the control C-O series and the treatment series at 25°C.

3D, 1W, 2W, and 3W: 3-day, 1-, 2-, or 3-week periods of aging.

Figure 3. The frequencies of nondisjunction in each brood of the treatment series at 10°C.

Table 1. Estimated number of eggs per female deposited during the pretreatment and treatment period before mating in high (25°C) and low (10°C) temperature.

Control and experimental series	Distribution of the estimated number of deposited eggs per female during the pretreatment and treatment periods (days)																								Total
	Pretreatment	Treatment																							
Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Control C-O	← 0.42 →																									0.42
3 days 25°C	← 0.09 →																									0.09
10°C	← 0.43 →																									0.43
1 week 25°C	← 0.3 →							← 0.06 →																		0.36
10°C	← 0.42 →							← 0 →																		0.42
2 weeks 25°C	← 0.47 →							← 2.97 →							← 0.96 →											4.40
10°C	← 0.56 →							← 0 →							← 0.006 →											0.56
3 weeks 25°C	← 0.27 →							← 1.13 →							← 1.54 →									← 0.56 →		3.50
10°C	← 0.12 →							← 0 →							← 0 →									← 0.003 →		0.12

Table 2. Nondisjunction data: control series, C-O and C. Nd: Nondisjunctional exceptions. Numbers without parentheses: both sexes jointly; numbers in parentheses: males only.

Broods (days)	Control series			
	C-O		C	
	Nd/Total	% Nd	Nd/Total	% Nd
1	2/ 3404	0.058	0/ 27	0.
	(2/ 1686)	(0.118)	(0/ 17)	(0.)
2	6/ 14672	0.040	4/ 12236	0.032
	(2/ 7393)	(0.027)	(3/ 6259)	(0.047)
3	9/ 21581	0.041	6/ 18135	0.033
	(8/ 10705)	(0.074)	(6/ 9069)	(0.066)
4	8/ 24104	0.033	5/ 19755	0.025
	(4/ 11892)	(0.033)	(5/ 9918)	(0.050)
5	8/ 26104	0.030	1/ 6001	0.016
	(7/ 12967)	(0.053)	(0/ 2905)	(0.)
6	4/ 22527	0.017	6/ 4783	0.125
	(2/ 11186)	(0.017)	(5/ 2350)	(0.212)
7	8/ 21706	0.036	2/ 4239	0.047
	(7/ 10752)	(0.065)	(1/ 2069)	(0.048)
8	9/ 19631	0.045	4/ 3191	0.125
	(7/ 9732)	(0.071)	(3/ 1519)	(0.197)
9	14/ 20668	0.067	8/ 5364	0.149
	(10/ 10335)	(0.096)	(7/ 2680)	(0.261)
10	13/ 17237	0.075	3/ 6060	0.049
	(9/ 8410)	(0.107)	(1/ 2998)	(0.033)
11	10/ 13872	0.072	0/ 3299	0.
	(7/ 6927)	(0.101)	(0/ 1623)	(0.)
12	8/ 10213	0.078	2/ 2486	0.080
	(5/ 4985)	(0.100)	(2/ 1220)	(0.163)
13	2/ 6311	0.031	6/ 3089	0.194
	(1/ 3068)	(0.032)	(1/ 1486)	(0.067)
14			2/ 3126	0.063
			(1/ 1456)	(0.068)
15			3/ 2539	0.118
			(1/ 1234)	(0.081)
16			0/ 2085	0.
			(0/ 1015)	(0.)
Totals	101/222039	0.045	52/ 96415	0.053
	(71/110038)	(0.064)	(36/ 47823)	(0.075)

Table 3. Nondisjunction data: high-temperature series (25°C).

Nd: nondisjunctional exceptions. Numbers without parentheses: both sexes jointly; numbers in parentheses: males only.

Broods (days)	Treatment series								Totals	
	3 days		1 week		2 weeks		3 weeks			
	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	% Nd	Nd/Total	% Nd
1	1/ 2891 (1/ 1353)	0.034 (0.073)	2/ 1513 (2/ 739)	0.132 (0.270)	2/ 1118 (1/ 537)	0.178 (0.186)	2/ 2321 (2/ 1150)	0.086 (0.173)	7/ 7843 (6/ 3779)	0.089 (0.158)
2	6/ 9730 (5/ 4891)	0.061 (0.102)	(4/ 6594 (2/ 3350)	0.060 (0.059)	4/ 6113 (4/ 3082)	0.065 (0.129)	1/ 8307 (0/ 4175)	0.012 (0.)	15/ 30744 (11/ 15498)	0.048 (0.070)
3	5/11015 (4/ 5371)	0.045 (0.074)	5/ 9293 (4/ 4683)	0.053 (0.085)	1/ 6783 (0/ 3335)	0.014 (0.)	4/14079 (3/ 6914	0.028 (0.043)	15/ 41170 (11/ 20303)	0.036 (0.054)
4	4/ 9267 (3/ 4548)	0.043 (0.065)	2/ 8027 (1/ 3945)	0.024 (0.025)	2/ 6225 (2/ 3099)	0.032 (0.064)	2/ 6700 (2/ 3294)	0.029 (0.060)	10/ 30219 (8/ 14886)	0.033 (0.053)
5	6/ 6761 (5/ 3286)	0.088 (0.152)	5/ 7418 (5/ 3714)	0.067 (0.134)	8/ 6723 (8/ 3255)	0.118 (0.245)	9/ 5001 (8/ 2498)	0.179 (0.320)	28/ 25903 (26/ 12753)	0.108 (0.203)
6-7	15/15910 (13/ 7628)	0.094 (0.170)	13/13447 (12/ 6428)	0.096 (0.186)	16/11047 (15/ 5184)	0.144 (0.289)	30/ 9609 (23/ 4584)	0.312 (0.501)	86/ 50013 (63/ 23824)	0.171 (0.264)
8-9	11/10702 (5/ 5045)	0.102 (0.099)	29/12511 (22/ 5887)	0.231 (0.373)	32/11871 (19/ 5753)	0.269 (0.330)	33/ 9074 (18/ 4354)	0.363 (0.413)	105/ 44158 64/ 21039	0.237 (0.304)
10-11	5/ 4705 (3/ 2284)	0.106 (0.131)	15/ 6906 (8/ 3367)	0.217 (0.237)	11/ 5187 (6/ 2449)	0.212 (0.244)	20/ 4790 (12/ 2335)	0.417 (0.513)	51/ 21588 (29/ 10435)	0.236 (0.277)
12-13	1/ 1163 (1/ 586)	0.085 (0.170)	3/ 1553 (2/ 760)	0.193 (0.263)	5/ 1021 (3/ 510)	0.489 (0.588)	3/ 1410 (2/ 702)	0.212 (0.284)	12/ 5147 (8/ 2558)	0.233 (0.312)
Totals	54/72144 (40/34992)	0.074 (0.114)	78/67262 (58/32873	0.115 (0.176)	81/56088 (58/27204)	0.144 (0.213)	104/61291 (70/30006)	0.169 (0.233)	329/256785 (226/125075)	0.128 (0.180)

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Table 4. Nondisjunction data: low-temperature series (10°C).

Nd: nondisjunctional exceptions. Numbers without parenthesis: both sexes jointly; numbers in parenthesis: males only.

Broods (days)	Treatment series: 3 days Nd/Total	3 days % Nd	1 week Nd/Total	1 week % Nd	2 weeks Nd/Total	2 weeks % Nd	3 weeks Nd/Total	3 weeks % Nd	Totals Nd/Total	Totals % Nd
1	9/ 1525 (4/ 728)	0.590 (0.549)	64/ 1246 (42/ 627)	5.136 (6.698)	49/ 604 (24/ 297)	8.112 (8.080)	8/ 144 (2/ 68)	5.555 (2.941)	130/ 3519 (72/ 1720)	3.694 (4.186)
2	6/ 8489 (5/ 4168)	0.070 (0.119)	3/ 4563 (2/ 2246)	0.065 (0.089)	2/ 4121 (2/ 2074)	0.048 (0.096)	5/ 7197 (4/ 3565)	0.069 (0.112)	16/ 24370 (13/ 12053)	0.065 (0.107)
3	5/13286 (4/ 6563)	0.037 (0.060)	7/10180 (5/ 5097)	0.068 (0.098)	7/10655 (7/ 5309)	0.065 (0.131)	20/21507 (20/10657)	0.092 (0.187)	39/ 55628 (36/ 27626)	0.070 (0.130)
4	4/ 8523 (3/ 4177)	0.046 (0.071)	6/ 8956 (6/ 4427)	0.066 (0.135)	2/ 8129 (1/ 3990)	0.024 (0.025)	(4/ 8869 (4/ 4483)	0.045 (0.089)	16/ 34477 (14/ 17077)	0.046 (0.081)
5	4/ 5651 (4/ 2753)	0.070 (0.145)	5/ 6121 (4/ 3003)	0.081 (0.133)	5/ 2688 (3/ 1317)	0.186 (0.227)	6/ 3609 (5/ 1834)	0.166 (0.272)	20/ 18069 (16/ 8907)	0.110 (0.179)
6-7	19/15143 (14/ 7144)	0.125 (0.195)	24/11787 (16/ 5583)	0.203 (0.286)	37/12379 (24/ 5857)	0.298 (0.409)	57/17632 (39. 8607)	0.323 (0.453)	137/ 56941 (93/ 27191)	0.240 (0.342)
8-9	12/10463* (.5/ 4970)	0.114 (0.100)	31/11750 (14/ 5678)	0.263 (0.246)	50/13919 (29/ 6705)	0.359 (0.432)	54/17456 (38/ 8581)	0.309 (0.442)	147/ 53588 (86/ 25934)	0.274 (0.331)
10-11	7/ 3201 (4/ 1583)	0.218 (0.252)	11/ 5587 (9/ 2735)	0.196 (0.329)	7/ 4141 (5/ 2011)	0.169 (0.248)	27/ 7085 (16/ 3423)	0.381 (0.467)	52/ 20014 (34/ 9752)	0.259 (0.348)
12-13	1/ 908 (0/ 472)	0.110 (0.)	1/ 1123 (0/ 560)	.0.089 (0.)	6/ 908 (5/ 460)	0.660 (1.086)	2/ 1723 (1/ 853)	0.116 (0.117)	10/ 4662 (6/ 2345)	0.214 (0.255)
Totals	67/67189 (43/32558)	0.099 (0.132)	152/61313 (98/29956)	0.247 (0.327)	165/57544 (100/28020)	0.286 (0.356)	183/85222 (129/42071)	0.214 (0.306)	567/271268 (370/132605)	0.209 (0.279)

*In this group accidental failure to count all the offspring resulted in actual counts of 8/6463 only. It was estimated that about 4 000 normal segregants and two of each of the nondisjunctional XXY and XO exceptions had been missed. These numbers were added to the initially recorded data.

Table 5. Probabilities from χ^2 tests for homogeneity between the control C-O series and the treatment series at 25°C.

Line	Compared broods (days)	Experimental series			
		C-O vs 3 days	C-O vs 1 week	C-O vs 2 weeks	C-O vs 3 weeks
1	1-13	0.002	<0.001	<0.001	<0.001
2	1- 4	0.5	0.4-0.5	0.7-0.8	0.4-0.5
3	5-13	<0.001	<0.001	<0.001	<0.001
4	1- 5	0.1-0.2	0.1-0.2	0.06-0.07*	0.3-0.4
5	6-13	0.002	<0.001	<0.001	<0.001
6	2- 4	0.4	0.5-0.7	0.9-0.95	0.2-0.3
7	5	0.08-0.09	0.2-0.3	0.009-0.01	<0.001
8	6- 7	<0.001	<0.001	<0.001	<0.001
9	8- 9	0.1-0.2	<0.001	<0.001	<0.001
10	10-13	0.5-0.6	<0.001	<0.001	<0.001

*male data only: 0.02

Table 6. Probabilities from χ^2 tests for homogeneity between the broods within each series.

Line	Temp (°C)	Compared broods (days)	Experimental series				
			C-O	3 days	1 week	2 weeks	3 weeks
1		1-4 vs 5-13	0.3-0.5	0.02-0.03*	0.001	0.001	0.001
2		1-5 vs 6-13	0.1-0.2	0.03-0.04†	0.001	0.001	0.001
3	25	1-2 vs 3-4	0.5-0.7	0.5-0.7	0.4-0.5	0.1-0.2	1.0
4		1 vs 2-4	0.8-0.9	1.0	0.3-0.5	0.1-0.2	0.3-0.4
5		1 vs 2-13	0.99	0.6-0.7	1.0	1.0	0.4-0.5
6		1 vs 2-4		0.001	0.001	0.001	0.001
7	10	1 vs 2-13		0.001	0.001	0.001	0.001
8		2-4 vs 5-13		0.002	0.001	0.001	0.001

*male data only: 0.07-0.09

†male data only: 0.1-0.2

Table 7. Probabilities from χ^2 tests for homogeneity between the control C-O and the treatment series at 10°C.

Line	Compared broods (days)	Experimental Series			
		C-O vs 3 days	C-O vs 1 week	C-O vs 2 weeks	C-O vs 3 weeks
1	1-13	0.001	0.001	0.001	0.001
2	2-13	0.001	0.001	0.001	0.001
3	2- 4	0.4-0.5	0.06-0.08*	0.5-0.6	0.01
4	5-13	0.001	0.001	0.001	0.001
5	1	0.001†	0.001	0.001	0.001
6	2	0.5-0.6	0.7-0.8	1.0	0.5-0.7
7	3	0.8-0.9	0.3-0.4	0.3-0.4	0.03-0.04
8	4	0.8-0.9	0.3-0.4‡	0.98	0.8-0.9
9	5	0.2-0.3	0.1-0.2	0.002§	0.002
10	6-7	0.001	0.001	0.001	0.001
11	8-9	0.04-0.05**	0.001	0.001	0.001
12	10-13	0.01-0.02††	0.004	0.001	0.001

*male data only: 0.02-0.03

†male data only: 0.1-0.2

‡male data only: 0.04-0.05

§male data only: 0.08-0.09

**male data only: 0.09-0.1

††male data only: 0.3

Table 8. Probabilities from χ^2 tests for homogeneity between the high- and low-temperature treatment series for the same period of treatment.

Line	Compared broods (days)	Experimental series			
		3 days	1 week	2 weeks	3 weeks
1	1-13	0.1-0.2	0.001	0.001	0.05-0.06
2	2-13	0.4-0.5	0.1-0.2	0.01-0.02	0.1-0.2
3	2- 4	0.98	0.3-0.4	0.5-0.7	0.004
4	1	0.001*	0.001	0.001	0.001
5	2	0.8-0.9	1.0	1.0	0.1-0.2
6	3	1.0	0.5-0.7	0.2-0.3	0.02-0.03†
7	4	1.0	0.7-0.8	1.0	0.95
8	5	0.95	1.0	0.6-0.7	0.8-0.9
9	6-7	0.3-0.5	0.02-0.03‡	0.01-0.02§	0.8-0.9
10	8-9	0.7-0.8	0.5-0.6	0.2-0.3	0.4-0.5
11	10-13	0.2-0.3	0.6-0.7	1.0	0.6-0.7

*male data only: 0.08-0.1

†male data only: 0.01-0.02

‡male data only: 0.2-0.3

§male data only: 0.2-0.3

Table 9. Analysis of the first-day broods from pair matings in the control C-O and in each experimental series. Number of P females classified according to the number of their offspring (see first column).

Number of F_1 per P ♀	Control C-O	Treatment series							
		3 days		1 week		2 weeks		3 weeks	
	25°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C
0	39	6	6	36	36	53	79	152	194
1	25	4	9	27	27 (1)	30	38 (3)	55	34 (2)
2	34	3	19	31 (1)	31 (1)	19	36 (2)	16	11 (4)
3	57 (1)	10	9	28 (1)	23 (7)	8	22 (5)*	7	3
4	54	10	15	11	15 (2)†	8 (1)	11	4	1
5	50	13	25 (1)	13	16 (4)‡	7	5 (1)	3	2
6	48	15	14 (3)	4	8 (2)	11	3 (2)§	1	1
7	28	10	17	7	6 (2)	3	1		
8	25	14	12 (3)	7	2 (1)	3	1		
9	22	15	10	4	1	3			
10	10	13	7 (2)	1		2		1	
11	7	9	5			1			
12	15	7	3	2		1			
13	11	7	1	2		2			
14	1	4	2						
15	4	1				1			
16	8	2		1					
17	1					1			
18		3							
19		1							
20	1								
21		2							
22		2							
25		1							
Total P ♀	440	152	154	174	165	153	196	239	246
Total F_1	2351	1243	850	523	413	398	278	155	85
Average F_1 per ♀	5.3	8.1	5.5	3.0	2.5	2.6	1.4	0.6	0.3
Total exceptions	1	0	9	2	20	1	13	0	6
% exceptions	0.042	0.	1.058	0.382	4.842	0.251	4.676	0.	7.058

Number of nondisjunctional exceptions is given in parentheses. Each exceptional fly came from a different mother except in the following four cases:
*two out of the listed five from one mother,
†two from one mother,
‡three out of the listed four from one mother, and
§two from one mother.

Table 10. Analysis of the second-day brood from those females which did not produce any viable offspring in the first-day "brood."

	Treatment series								
	C-O	3 days		1 week		2 weeks		3 weeks	
	25°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C
Fertile P females	30	5	3	26	28	40	50	113	157
F_1	807	269	165	598	698	1206	1084	2077	2767
Exceptions	0	0	0	0	0	1	1	0	2
% exceptions	0	0	0	0	0	0.08	0.09	0	0.07

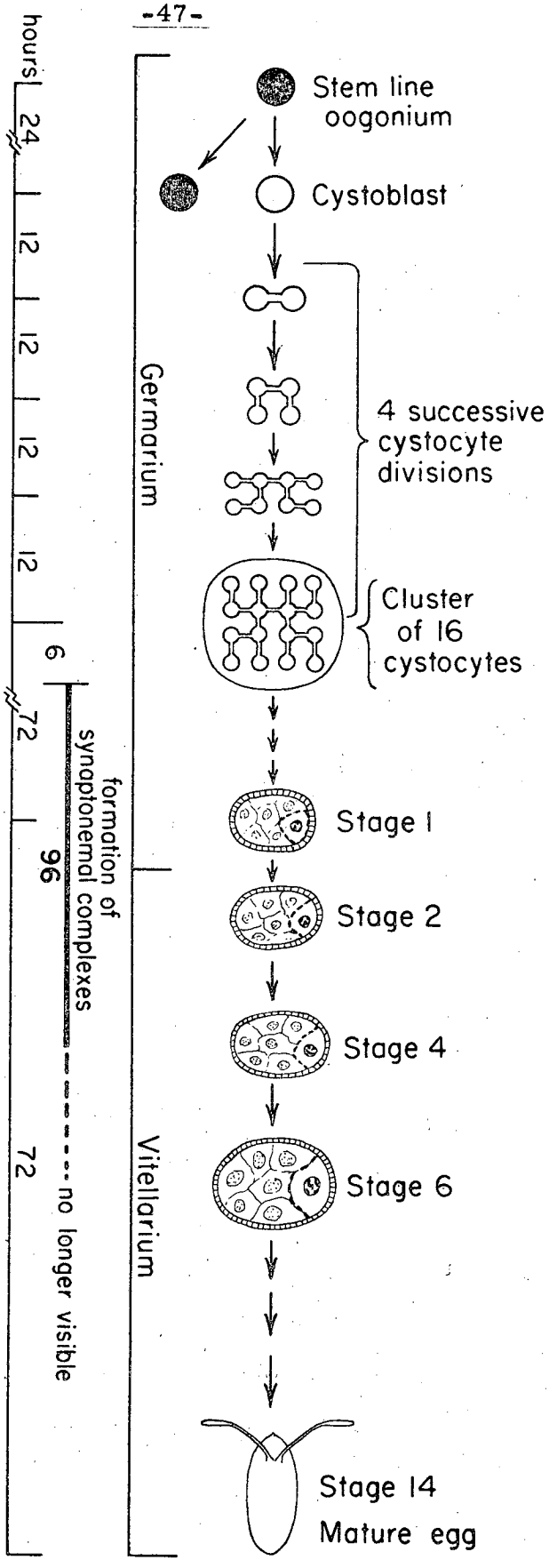

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Figure 1.

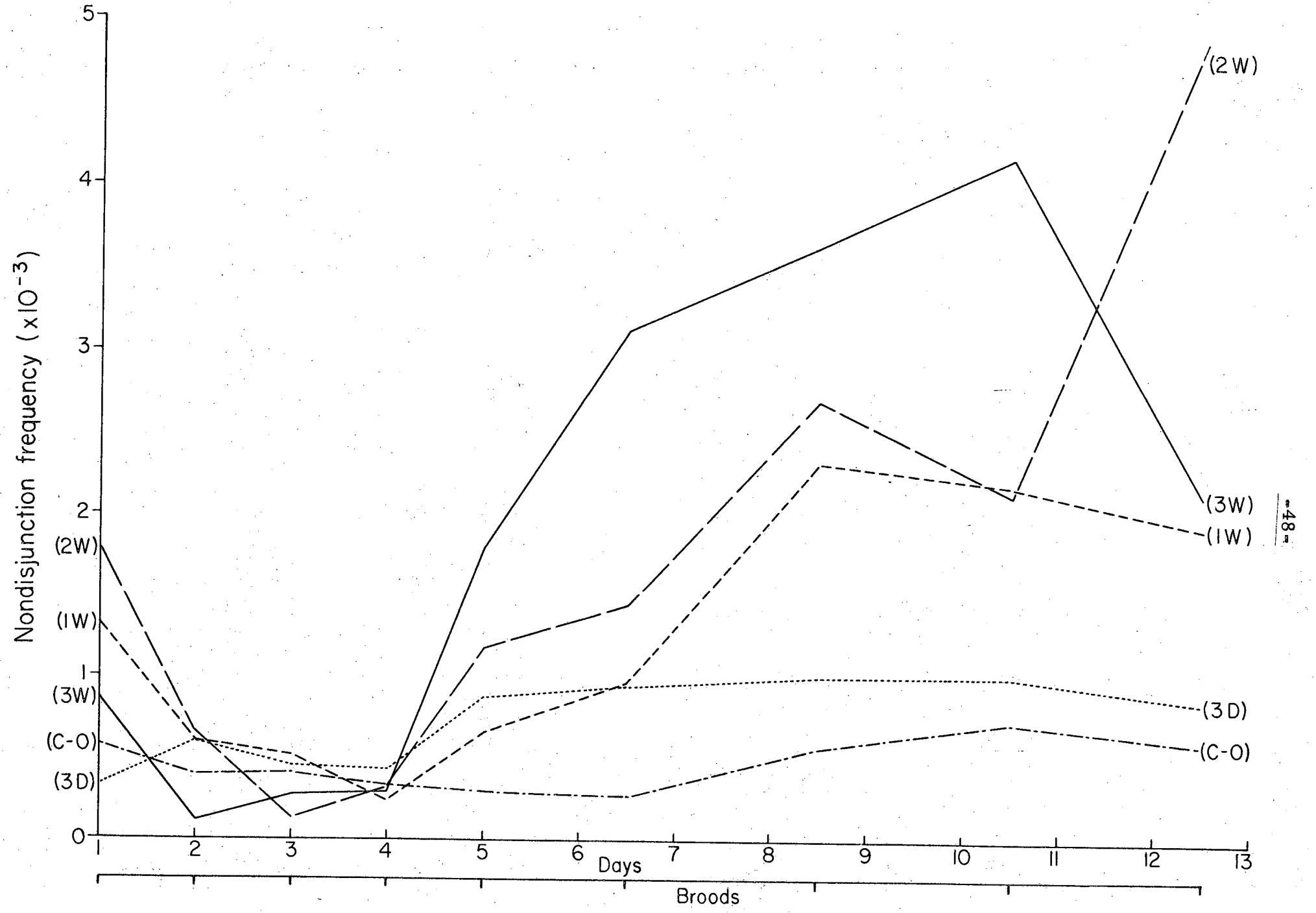

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Figure 2.

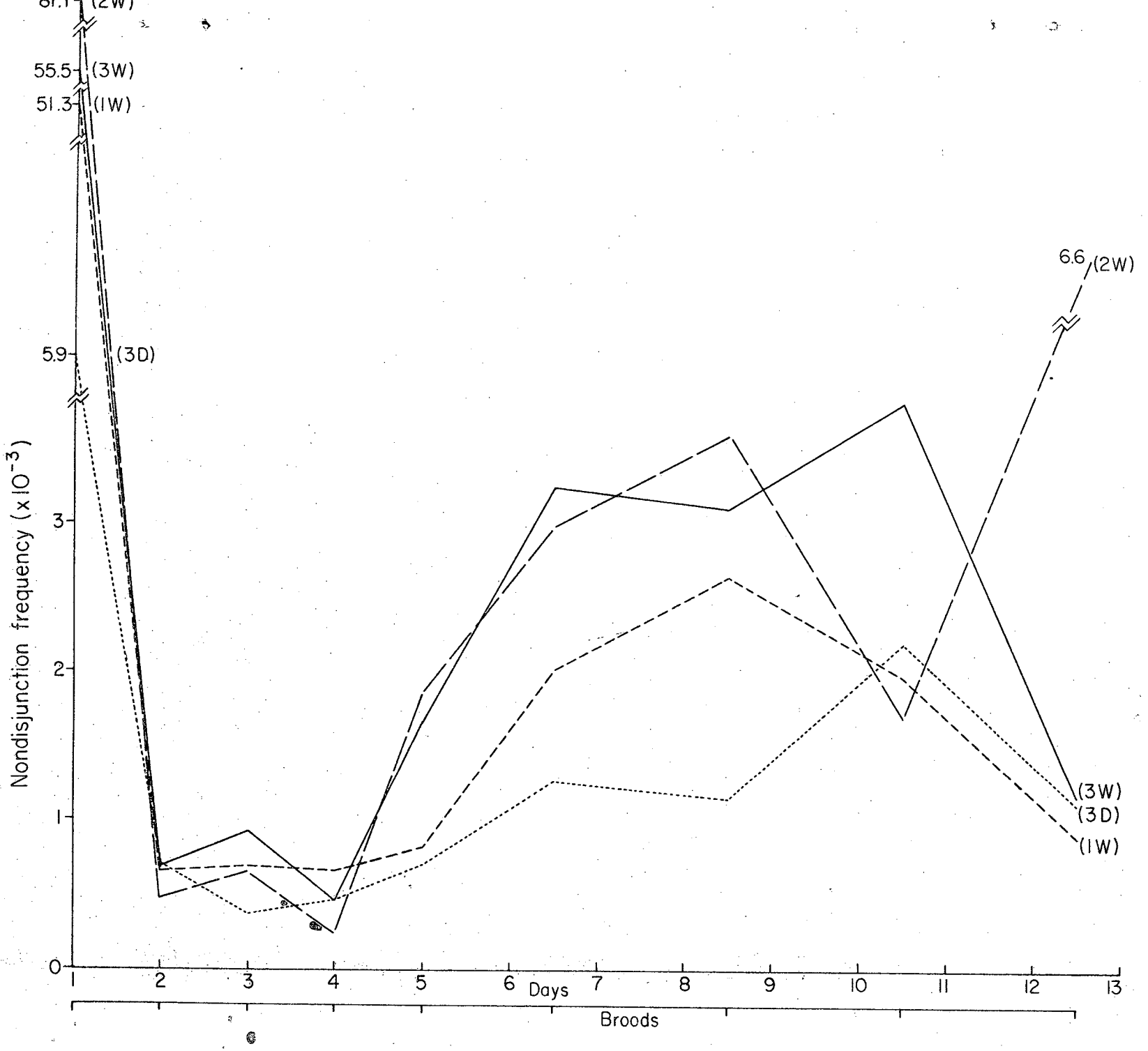

Figure 3.

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