The Effect of Sequence Homozygosity on the Frequency of X-Chromosomal Exchange in Drosophila melanogaster Females

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ABSTRACT

The repair of mismatched heteroduplex DNA has been implicated in the normal resolution of meiotic exchange events. Although sequence microheterogeneity over defined intervals of homologous chromosomes has been correlated with local effects on recombination, this correlation has not previously been extended to effects on chromosomal levels of exchange. In order to determine the role of microheterogeneity in normal exchange between homologs, a system was devised for monitoring exchange between isogenic X chromosomes. Lack of microheterogeneity did not significantly alter the frequency of exchange along the isogenic X chromosomes relative to controls or to previously reported values. There were, however, characteristic levels of exchange intrinsic to the cloned X chromosomes in each of the lines tested.

One is always concerned that the way a biological phenomenon is measured influences the outcome. Recent observations on meiotic crossing over have raised the possibility that total chromosome levels of exchange might be influenced by the frequency of microheterogeneities between the two homologs involved. Ordinary experiments using marked chromosomes to measure chromosomal levels of crossing over do not address this issue, because the markers themselves contribute heterogeneity in a background of presumptive homogeneity.

It has long been known that, at the fine structure level, the frequencies and kinds of recombination events recovered are affected by the nature of the mutations used to monitor them. Collectively, these effects have been termed "marker effects." It has relatively recently become obvious that chromosomes accumulate micro changes in sequence at surprisingly high rates. For example, in Drosophila, different stocks are so likely to have accumulated sequence differences that correlating the phenotype of a mutation with changes at the DNA level is nearly impossible unless the original chromosome on which that mutant was induced is available for comparison. Consequently, any two heterozygous chromosomes are now expected to differ at many more sites than those that have been deliberately introduced to monitor crossing over. No degree of backcrossing will ever render those two chromosomes homozygous for all sites since there will always be a residuum around the markers as well as the markers themselves.

There are two independent reasons for being concerned about the possibility that sequence heterozygosity affects exchange probability. The first derives from the two aspects of the recombinational phenotype of mei-9 mutations in Drosophila (BAKER and CARPENTER 1972; CARPENTER 1982). First, although the frequency of formation of meiotic recombinational heteroduplexes is at least equal to controls, in mei-9 females these heteroduplexes are not repaired but rather are resolved by replication, giving rise to mosaic progeny. In addition, the cell total of exchange events is drastically reduced, giving rise to high frequencies of chromosomes that have no exchanges and therefore fail to segregate normally at anaphase I. The fact that two independent mei-9 mutant alleles show both recombinational defects implies that both defects are due to misbehavior of the same presumptive protein. The simplest hypotheses involve only one time and action of the wild-type allele to ensure that both processes go normally. These are hypotheses in which the two effects of mei-9 are either the result of one simultaneous defect or else one defect is an unavoidable result of the other (that is, the two kinds of effects represent dependent events). One possibility is that repair of mismatched base pairs is an essential step in the generation of a meiotic exchange event; in the absence of repair an event can resolve only as noncrossover. If this be the case, then in wild type the normal level of exchanges is being assured by microheterogeneities between the homologs which serve as triggers for repair when they are crossed by recombinational heteroduplex, and, if the chromosomal level of heterozygosity is relatively low, the meiotic recombination machinery must be able to find the few heterozygosities efficiently. Although this is not an attractive possibility, it cannot be dismissed out of hand. Moreover, it permits a straightforward predic-
tion: in *mei-9* females, exchange between completely homozygous chromosomes should be greatly reduced or absent.

The second observation linking sequence heterozygosity and exchange probability is that "marker effects" reduce the probability of exchange in a marked (short) interval, as most elegantly shown in yeast by Borts and Haber (1987). When the number of heterozygous sites was high the exchange outcome decreased twofold, although the frequency of recombinational events in the interval remained constant. Although Borts and Haber interpret their results to indicate that exchanges occur at the expected frequency but tend to be rendered undetectable as such because of additional, repair-stimulated recombinational events that involve the duplicated flanking regions peculiar to the genotypes used to monitor recombination in this region, it is also possible that events that would have proceeded to exchange in the absence of heterologies abort into alternate outcomes prior to actual exchange. This suggests to us an alternative hypothesis for the effect of heterozygositides: that they may tend to inhibit the exchange outcome in their vicinity implying that exchanges are more likely to arise from intervals that are completely homologous. If so, then completely homozygous chromosomes are predicted to exhibit a level of exchange that is equal to or higher than the frequency observed with chromosomes that are heterozygous for a few scattered marker mutations.

Meiotic exchange events have the immediate effect of being the physical basis for chiasmata, and chias mata in turn are essential for regular reductive segregation of homologs at anaphase I. As long as a pair of homologs is able to accomplish one exchange event, anywhere along their joint length, disjunction will be regular. There are general cellular functions that regulate both the numbers and chromosomal patterns of exchanges. This system has compensatory aspects that have been described, although the mechanism is not understood; an alteration of the exchange frequency in one region is generally accompanied by the opposite change in others [for example, the interchromosomal effect, which also acts intrachromosomally, see Lucchesi and Suzuki (1968) for review]. Consequently, although one could build up inferences from studies of regions it is more informative to examine effects of heterozygosity vs. homozygosity for an entire chromosome.

Exchange between homozygous chromosomes cannot be monitored directly, since complete homozygosity precludes the presence of markers. However, since the chromosomal rate of exchange averages only 1.3 events per X per meiosis in Drosophila, we can monitor the converse, that is changes in the frequency of meioses in which the X chromosomes have no exchange anywhere along their length (are E₀). In most organisms, complete failure of exchange leads to failure of chiasma formation and failure of regular reductive segregation at anaphase I. Drosophila females, however, have a backup system, the distributive system, which normally insures that even in the meioses where the X's fail of exchange (around 5% of meioses, in experiments with standard marked X's) they nevertheless exhibit proper reductive segregation. Moreover, in the presence of an additional, heterologous chromosome (specifically, the Y chromosome), the distributive system now operates to send both nonexchange X chromosomes to one anaphase I pole and the Y to the other (see Figure 2A). There is no effect on the reductive disjunction of X chromosomes that have succeeded in exchange. This effect of a supernumerary Y chromosome was first investigated by Bridges (1916) and termed "secondary nondisjunction"; that it is an attribute of the distributive system was elegantly demonstrated by Grell (1962a, b). Distributive segregation is a normal segregation system in the sense that chromosomes under its aegis move predictably and regularly to the anaphase I poles, without the chromosome loss or other misbehavior that, in other organisms, complicate measuring the frequency of meioses in which a pair of homologues are nonexchange. Although probably not quite all E₀ X's segregate from the Y in XXY females and a few X bivalents with a single, distal exchange do [see Hawley (1988) for a review], these are minor effects. We can therefore use the frequency of secondary nondisjunction in XXY females, isogenic and nonisogenic for the X chromosomes, to measure the frequency of meioses in which the entire X is E₀ and therefore, by subtraction, the frequency with which those X's undergo exchange.

We cloned out a number of independent X chromosome isolates from each of three different stocks by crossing individual males to a common balancer-XXY stock (see Materials and Methods); the resulting isosequential XXY females (and their XX sibs) were tested immediately to minimize the chance of those presumptively isosequential X's accumulating microheterogeneities from spontaneous events. Rates of secondary nondisjunction in these isogenic lines were close to the generally accepted value (5%) and also to the internal control (heterozygotes between lines), so it does not appear that microheterogeneity is necessary for exchange in Drosophila. However, to our surprise the different X chromosomes consistently each exhibit a characteristic frequency of secondary nondisjunction, hence basic level of exchange. Since stock synthesis involved crossing to a common stock, and since (with one single exception) all sublines from each of the three source stocks are congruent, these differences reflect intrinsic X-linked differences be-
tween these chromosomes in their responsiveness to the general cellular meiotic exchange process, a phenomenon that was not expected.

MATERIALS AND METHODS

All crosses were done at 25° on standard cornmeal-molasses-agar medium with propionic acid as mold inhibitor. Test crosses, each of which involved one virgin female plus several males, were done in shell vials. The parents were transferred to fresh food on day 5 (day of set up = day 0) and removed 5 days later. The progeny from each of the two vials were counted on that vial's days 12, 14 and 17. For descriptions of markers and the balancer FM6, see LINDSLEY and GRELL (1986) and LINDSLEY and ZIMM (1985, 1986, 1987).

Stock construction: The lines, each completely isogenic for a different X chromosome, were established as follows (Figure 1). Three different basic marked-X stocks were selected by three criteria: they carried 

\[
y \text{cin} \ w/\text{FM6} + y'Y \] 

x single 

\[
y/\text{FM6} + y'Y \] 

\[
y/\text{FM6} + y'Y \] Stock

Figure 1.—Mating scheme used to generate isogenic XXY stocks.

\[
\begin{align*}
y \text{cin} \ w/\text{FM6} + y'Y & \quad \text{single} \ y/Y \\
y/\text{FM6} + y'Y & \quad y/y'Y \\
y/y'Y & \quad \text{stock}
\end{align*}
\]

There were a few unanticipated complications. A total of 87 P1 single-male crosses were set up; these resulted in only 25 isogenic-X stocks. 21/33 of the F1 y crosses were sterile, as were 15/33 w w f36a and 4/21 y pm. This was a high frequency of sterility even given that they were single-male crosses of yellow males (which are reputed to have lower mating success than wild type) to, mostly, single females. (Fertility of the single-pair F1 matings and all subsequent generations was high.) Furthermore, an additional 6, 7 and 9 crosses gave only \( y \text{cin} \ w + y'Y \leftrightarrow \text{FM6} \) segregations in the F1, as though in these females \( y' \) (± the Y) were attached to the \( y \text{cin} \ w \). This exclusive type of segregation was also observed in some crosses within the \( y \text{cin} \ w/\text{FM6} \) stock. The remaining 6, 11 and 8 crosses gave the expected segregations for X/Balancer females: since Balancer heterozygosity reduces the frequency of exchange, the majority segregation type is \( X + \text{Balancer} \leftrightarrow Y \); however, enough X \( 
\]

\[
\begin{align*}
\text{FM6} & \quad \text{y'Y stock} \\
\text{FM6} & \quad \text{y'Y stock} \\
\text{FM6} & \quad \text{y'Y stock}
\end{align*}
\]

\( 
\]

\[
\begin{align*}
\text{FM6} & \quad \text{y'Y stock} \\
\text{FM6} & \quad \text{y'Y stock} \\
\text{FM6} & \quad \text{y'Y stock}
\end{align*}
\]

The lines, each completely isogenic for a different X chromosome, were established as follows (Figure 1). Three different basic marked-X stocks were selected by three criteria: they carried \( y \text{cin} \), necessary for scoring segregation of the X's and Y's in the test crosses; they were healthy at the time and had not been noted to have suffered breakdown or contamination problems during their carrying history; and they were already available in the stock collection. The three stocks are: \( y/Y \), otherwise unmarked; \( y \text{pm}/Y \), \( \text{spa}^a \); and \( y \ w f36a \). All of these stocks have been maintained in La Jolla for at least 10 yr. The first two originated from the collection of LARRY SANDEL; the \( y \ w f36a \) stock was acquired years ago for studies of somatic crossing over. It is unlikely that any of these stocks are recently related.

The plan had been to make isogenic-X stocks with a marked Y chromosome and wait for the occasional spontaneous primary nondisjunction event to generate XXY stocks for each. However, fortuitously a balanced stock with a marked Y happened to be segregating high frequencies of XXY females so this stock was used to isolate single X's and simultaneously to introduce the marked Y. [In this stock, \( y \text{cin} \ w/\text{FM6}, y^2 \text{dm} B \) in which the males carry the \( y'Y \). XXY females that are \( y \text{cin} \ w/y \text{cin} \ w/y'Y \) were selected when they occur spontaneously because they are more fertile than the other genotypes; \( \text{cin} \) is a resuable maternal-effect lethal mutation and the \( y'Y \) carries \( \text{cin} \) (BAKER 1973.)] From each of the original three marked-X stocks at least 10 individual males (alive, therefore their X's carried no lethals) were crossed separately to \( y \text{cin} \ w/\text{FM6} \) virgin females; within each of these crosses, all progeny that received a paternal X chromosome received the same X, barring spontaneous events. Within each of the (10 x 3) lines, individual virgin \( yX/\text{FM6} + y'Y \) females were crossed to individual \( yX/y'Y \) brothers to establish several sublines and each of these was virgined to eliminate FM6 the next generation. Stocks were kept by selecting XXY females until they could be tested, which was from one to four generations after homzygosity was attained. Control heterozygous females were generated by crossing one \( y \text{pm} \) line to one \( y \ w f36a \) line.
RESULTS

For each of the lines tested, experimental (XXY) and control (XX) females were each crossed to two types of males, attached-XY (XYp,Yb/0) and free XY (y/ B'Y) to control for differential viability of exceptional and regular progeny. Although Y chromosome hyperploidy is relatively innocuous in a single extra dose, two extra Y's do reduce viability; moreover, in these crosses the Y's must be marked so that they can be followed and those markers, being small duplications of X segments, themselves reduce viability. Crossing to both XY and X/Y males controls for these sources of differential viability, since each female gamete class yields progeny of normal viability in at least one cross (Table 1).

In all of the parents, the majority of meioses are expected to yield regular sex-chromosome segregation (X ↔ X in females; X ↔ Y in free X/Y males), which will in turn yield regular progeny ("R" in Table 1; Figure 2). These crosses were designed to measure the frequency of distributive (XX ↔ Y) segregation, which will produce exceptional progeny ("E" in Table 1, Figure 2); distributive segregation is expected to be a relatively frequent event. However, the sex chromosomes in all types of females and in the X/Y males can occasionally entirely fail of segregation, that is, undergo primary nondisjunction (XX(Y) ↔ 0 in females, XY ↔ 0 in X/Y males—the XY, of course, always segregates XY ↔ 0). The progeny that result from primary nondisjunction ("mat" and "pat" in Table 1) are phenotypically recognizable in most crosses, although not all classes were consistently different from the much more frequent regular progeny types or the occasional left-over parent. Consequently, although primary nondisjunction was detected, the apparent frequencies are probably slight underestimates.

The summed data are presented by progeny phenotype in Table 2 and the gametic frequencies of the various types of segregations are tabulated in Table 3. Exceptional female gametes are recoverable only when fertilized by one type of sperm, whereas regular gametes are recoverable when fertilized by either (Figure 2B). To compensate for this and also to match Y aneuploidy as closely as possible between exceptional and regular progeny types, the formulas in Table 1 were used to calculate the frequencies of XX ↔ Y distributive disjunction and of XX ↔ 0 nondisjunction.

Primary nondisjunction frequencies are, as expected, low in both males and females (Table 3; "standard" values are ca. 0.1% for both males and females [Baker and Carpenter, 1972]); the frequencies of distributive segregations in XXY females (XX ↔ Y) are also within the normal range (ca. 5%, see Carpenter, 1973). However, unexpectedly, the different isogenic lines differ, giving values that range from 3% to 8% (Table 3). This might reflect intrinsic differences between the starting stocks or it might reflect variation in levels of retention of the complete homozygosity that is the issue being addressed. We therefore analyzed the data by line and by progeny from individual females in order to determine the source of these differences.

Since these are single-female crosses, there is quite a bit of variability within lines in total number of progeny per female tested; moreover, since the frequency of secondary nondisjunction is low, there is also high relative variance in number of exceptional progeny per female. There are a number of ways to address the significance of this between-cross variability; the simplest is visual, and single-female nondisjunction frequencies are plotted by line in Figure 3. Crosses which yielded fewer than 11 relevant progeny are not plotted, although they have been included in the sums of Tables 2 and 3. Lines for which more than one symbol are used had more than one subline tested; in each case, the sublines were tested on different dates. Moreover, the different lines from a parent stock were tested on different dates—for each of the parent stocks, the line tests covered the interval from generation one of homozygosity to generation four.

It is obvious from Figure 3 that the crosses within isogenic lines derived from the y and y pn stocks are no more variable than expected from sampling error.
has been done for Table parent stock can be considered to be samples from and also from statistics (95% Poisson ranges on ob-
ter from y
from the three parent stocks reproducibly yield dif-
ferent frequencies of secondary nondisjunction and
enough, given the necessary limit postulate for this hypothesis: that the recombination machinery is likely
to find a single heterozygosity present anywhere along the X. However, since the lines were kept with multi-

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>y'B2</th>
<th>y'd</th>
<th>y'Bd</th>
<th>y'B2</th>
<th>pat</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXY</td>
<td>XXY</td>
<td>X0</td>
<td>[XXY]</td>
<td>[XY]</td>
<td>XXY</td>
</tr>
<tr>
<td>(R)</td>
<td>R</td>
<td>Mat*</td>
<td>Mat</td>
<td>R</td>
<td>R*</td>
</tr>
</tbody>
</table>

R = regular maternal segregation; (R) indicates a class of low viability and R indicates a class utilized in calculations of segregation frequency.
F = distributive (XX ↔ Y) maternal segregation.
Mat and Pat = maternal or paternal nondisjunction (XXY ↔ O, XX ↔ O, and XY ↔ O).
* indicates classes that will exhibit the maternal markers pn or w f36a in the appropriate crosses.
For XXY × XY crosses, XX ↔ Y segregation frequency is calculated as: \( (E + E)/(E + E + R + R) \); for XXY × XY crosses, it is calculated as: \( (E + E)/(E + E + R + R) \); for both types of XXY crosses, maternal nondisjunction is calculated as: \( (R + Mat)/[R^2(R + R + Pat) + (Mat + Mat)] \); paternal nondisjunction is calculated as Pat/Total.

### DISCUSSION

The initial question posed by these experiments is resoundingly answered: as measured by the frequency of nonexchange X chromosomes that segregate distributively from a Y chromosome, the frequency of exchange along the entire X chromosome is neither greatly increased (lower secondary nondisjunction) nor decreased (higher secondary nondisjunction) when the two X chromosomes are completely isogenic. Isogenic X’s gave 3–8% secondary exceptions, “standard” nonhomogeneous X chromosomes yield 5–10% secondary exceptions (see, e.g., CARPENTER 1973), and the control heterozygous cross reported here yielded 7% (Table 3; Figure 3). It is therefore clear that microheterogeneity is not necessary for exchange events to occur in Drosophila; moreover, the ca. 5% of nonexchange X tetrad that occur in normal heterozygous crosses are not the result of failure of some events due to microheterogeneity since this range of distributive disjunction continues to occur when the X’s are isogenic.

There are two kinds of concerns. The first is that, although each of the lines tested does represent the Drosophila equivalent of a cloned chromosome (having X chromosomes all of which were derived from the new X in the egg which gave rise to the founding father), nevertheless those chromosomes have at a minimum gone through four flies’ worth of mitotic divisions and three meioses before being tested, and if spontaneous generation of microheterogeneity be very rapid then the “isogenic” chromosomes may be heterogeneous. (One might think that one mutational change during the generation of each line would be enough, given the necessary limit postulate for this hypothesis: that the recombination machinery is likely to find a single heterozygosity present anywhere along the X. However, since the lines were kept with multi-

### FIGURE 2

A. Anaphase I behavior of exchange (left) and nonexchange (right) X chromosomes in the presence of a Y chromosome. B. Punnett square for regular and distributive segregation in XXY females.

(actual error bars are not shown for simplicity); moreover, the different isogenic lines from a given parent stock are also consistent with each other. One line from y w f36a is clearly segregating two types of females with respect to XX ↔ Y segregation frequency (Figure 3; Tables 2 and 3), to be discussed below; the other lines are not, and again the different lines are consistent with each other. Both from this visual inspection and also from statistics (95% Poisson ranges on observed exceptional progeny), except for the one unusual y w f36a line, the different isogenic lines from a parent stock can be considered to be samples from the same population and the data can be summed, as has been done for Table 2. Isogenic X chromosomes from the three parent stocks reproducibly yield different frequencies of secondary nondisjunction and therefore appear to have intrinsically different frequencies of total chromosomal exchange.
TABLE 2

Results of the crosses of y (pn) (w f)/y (pn) (w f)/(2y+/Y) females by $\bar{X}Y$, y $B/O (=\bar{X}Y)$ or by yB'/Y (= X/Y) males

<table>
<thead>
<tr>
<th>Cross</th>
<th>XXY Females</th>
<th>XX Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yB? y6 y'? yB6</td>
<td>yB? y6 y'? yB6</td>
</tr>
<tr>
<td>$y \times \bar{X}Y$</td>
<td>59 567 0 0</td>
<td>517 655 1 10</td>
</tr>
<tr>
<td>$y \times X / Y$</td>
<td>0 0 369 355</td>
<td>14 17 381 138</td>
</tr>
<tr>
<td>y pn $\times \bar{X}Y$</td>
<td>302 1329 0 2</td>
<td>1029 1225 59 43</td>
</tr>
<tr>
<td>y pn $\times X / Y$</td>
<td>0 1 711 746</td>
<td>21 36 810 548</td>
</tr>
<tr>
<td>$y w f \times \bar{X}Y$</td>
<td>273 1545 0 0</td>
<td>1313 1676 142 76</td>
</tr>
<tr>
<td>$y w f \times X / Y$</td>
<td>0 0 2032 1704</td>
<td>208$^b$ 187 2029 1559</td>
</tr>
</tbody>
</table>

y floor 5 $\times \bar{X}Y$
low; 4 v. 22 112 0 0 105 147 17 7 — 420
high; 2 v. 0 23 0 0 21 19 24 13 — 100
$X / Y$
low; 6 v. 0 0 92 92 11 6 85 73 1$^e$ 360
high; 3 v. 0 0 27 19 30 23 15 20 1$^e$ 135
$y pn / y w f$
$\times \bar{X}Y$ 76 629 0 0 395 611 40 27 — 1728 1046 1258 1 0 — 2505
$X / Y$ 0 0 429 367 33$^f$ 35 424 278 0 1566 1 1 1282 998 0 2282

Y line 5 $\times \bar{X}Y$

$^a$ Paternal nullo gamete (male progeny with only maternal markers).
$^b$ Paternal diplo gamete (female progeny with both paternal sex chromosome markers).
$^c$ Includes one gynandromorph.
$^d$ y w f female.
$^e$ Includes one equational exception (y w B$^f$ female).
$^f$ — not applicable or not recognizable.

TABLE 3

Frequencies of the various segregations for the data from Table 2

<table>
<thead>
<tr>
<th>Maternal Genotype</th>
<th>XXY Female</th>
<th>XX Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LX $\leftrightarrow$ Y segregations</td>
<td>Mat. XXY $\leftrightarrow$ O</td>
</tr>
<tr>
<td></td>
<td>No. Percent</td>
<td>No. Percent</td>
</tr>
<tr>
<td>y/y</td>
<td>52/1915 2.0 2.7 3.5</td>
<td>0</td>
</tr>
<tr>
<td>y pn/pn</td>
<td>159/4014 3.4 4.0 4.6</td>
<td>3/4014 0.07</td>
</tr>
<tr>
<td>y w f/y w f</td>
<td>613/7572 7.5 8.3 9.1</td>
<td>0</td>
</tr>
<tr>
<td>y pn/y w f</td>
<td>135/2021 5.6 6.7 7.8</td>
<td>0</td>
</tr>
<tr>
<td>y w f line 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Low&quot;</td>
<td>41/468 6.2 8.8 11.8</td>
<td>0</td>
</tr>
<tr>
<td>&quot;High&quot;</td>
<td>90/178 40.1 50.6 61.6</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ The bold-face number is the observed gametic frequency of XX $\leftrightarrow$ Y segregations; the bounding values are Poisson 95% confidence limits, calculated from the number observed using the tables in CROW and GARDNER (1959) (y w f extrapolated).

ple parents after generation two, any single mutational change is expected to be heterozygous in a maximum of 50% of the tested females; at least 20 different chromosomes must be segregating in the tested line for the probability of homozygotes to be less than 5%.)

Moreover, perforce there is one fly generation + meiosis involving a heterozygous homologue. Although FM6 is not an ideal balancer (there are very infrequent double crossovers in FM6+/ heterozygotes) it is certainly adequate here to insure that virtually all of the chromosomes tested were non-crossovers; however, it is possible (even likely) that simple gene conversion events continue to occur (see, e.g., CHOVNICK 1973). Since we do not know the chromosomal frequencies of simple gene conversion events even in isosequential X's we can only estimate this contribution to contamination. Since the X is 66 mu long, the mean probability of a crossover per entire X is 0.66; if simple gene conversion (gene conversion without a concomitant exchange) be equal in frequency to crossing over, then the mean probability of simple gene conversion per entire X is also expected to be 0.66, and of those only half (0.33) are expected to be convertants from FM6 to the X being cloned. The number distribution of exchange events between meioses is not binomial or Poisson, but that of simple gene conversion events might be; if so, the expected distribution of contaminated chromosomes is: 0.72 no simple conversions, 0.24 one simple con-
version event somewhere along the X, and 0.04 with 2 or more simple gene conversion events. Consequently, unless simple gene conversion is much more frequent than exchange, most of the cloned X's are expected not to be at risk of contamination from this source.

However, the issue of rapid generation of microheterogeneity does need to be addressed, especially since there were at least two instances of mutations detected: the single sn mutation in one of the y pm lines and line 5 of y w f^36e, which segregated for high secondary nondisjunction (and low fertility), see Table 3 and Figure 3. Unfortunately, the segregation in line 5 was not noticed at the time, so its cause can only be inferred; however, high secondary nondisjunction accompanied by normal primary nondisjunction is the expected behavior of inversion heterozygosity (Grell 1962a, b), and an unrecorded y w f^36e line did have a segregating inversion detected during the analysis of the sn mutation (see MATERIALS AND METHODS), so it is possible that there was one single spontaneous inversion event, just as there was one single spontaneous sn mutation. These are not unusually high frequencies, given the numbers of flies examined during the course of the stock building, but that any were detected does mean that undetected microheterogeneity might have occurred (by some process other than one associated with hybrid dysgenesis, see MATERIALS AND METHODS) at a high enough frequency to invalidate the presumed isogenicness of the cloned chromo-

FIGURE 3.—Frequencies of XX ↔ Y segregation per tested female for each of the different tested lines. Calculations followed the formula in Table 1; vials with fewer than 11 relevant progeny are not plotted. Open symbols (above the line) represent frequencies from vials that did yield at least one exceptional progeny; filled symbols (below the line) represent the frequency that would obtain if one exception had been observed in those vials that in fact gave none. X's indicate frequencies that are significantly higher than the rest of the vials for that line. When more than one subline was tested, they are given distinctive symbols. A, y pm/y pm/y+Y; B, y pm/y w f^36e/y+Y; C, y/y+Y; D, y w f^36e/y+Y.
somes. However, the facts that 1) chromosomes from the three different stocks do differ in secondary nondisjunction frequency and 2) with the exception of 8\( \Delta 36a \), the various lines from a stock do not differ in secondary nondisjunction frequency (see Figure 3) mean that if these lines are exhibiting "standard" secondary nondisjunction frequencies because of acquired microheterogeneity then each of the lines from a specific stock has acquired the same level—an assumption that seems unreasonable. And finally, there is the unanticipated finding that the different starting stocks have different intrinsic frequencies of secondary nondisjunction. Since each of the lines was outcrossed to the same fourth stock, the autosomal backgrounds are expected to be similar; consequently, the differences are inherent in the X chromosomes themselves. There are two kinds of causes of the different frequencies of secondary nondisjunction: either the X's differ with respect to the proportion of 8\( O_x \)'s that disjoin distributively from the Y or else they differ in the frequency of nonexchange tetrads. Differences in 8\( O_x \) frequencies could arise in two different ways which cannot be distinguished here: either the chromosomal total frequencies of exchange could be different or the exchange frequencies could be identical but the distributions of those exchanges across the different tetrad types (single exchange, double exchange, etc.) could be chromosome specific. Moreover, there are two kinds of causes: differences in 8\( E_x \) frequencies could either be due to differences in the substrate (the base sequences along the X chromosomes) or to differences in (X-linked) genes having minor effects on the recombinational system itself. Such genetic variants are common among chromosomes isolated from natural populations (Lindsley et al. 1968) when the test is for trans effects, so the possibility that the cis effect seen here results from the same kind of minor perturbation of the exchange-generating system cannot be eliminated. However, the observation that the different chromosomes appear to display codominance in secondary nondisjunction frequency (8\( \Delta n / \Delta w f \) females; Tables 2 and 3) means that exchange frequency and/or tetrad distribution may indeed respond to details of base sequence even in the presence of isosequentiality.

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**LITERATURE CITED**


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