COMPOUND AUTOSOMES IN *DROSOPHILA MELANOGASTER*: THE MEIOTIC BEHAVIOR OF COMPOUND THIRDS[1]

DAVID G. HOLM[2,3] AND ARTHUR CHOVNICK

Genetics and Cell Biology Section, The Biological Sciences Group, The University of Connecticut, Storrs, Connecticut 06268

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ABSTRACT

Studies of the meiotic distribution of compound-3 chromosomes in males and females of *Drosophila melanogaster* provided the following results. (1) From females homozygous for the standard arrangement of all chromosomes other than *C(3L)* and *C(3R)*, less than 5% of the gametes recovered were nullosomic or disomic for compound-3 chromosomes. The frequency of nonsegregation differed between strains, but within a given strain it remained relatively constant. (2) According to egg-hatch frequencies, *C(3L)* and *C(3R)* segregate independently during spermatogenesis. (3) In females, structurally heterozygous second chromosomes occasion a marked increase in the recovery of nonsegregational progeny; in males, rearranged seconds have no apparent influence on the distribution of compound thirds. (4) The highest frequencies of nonsegregational progeny were recovered from *C(3L);C(3R)* females carrying compound-*X* (plus free *Y*) chromosomes. (5) In comparing the recovery of nonsegregating compound thirds to the recovery of rearranged heterologs, a definite nonrandom distribution was realized in several crosses. These results are examined in reference to the concepts of distributive pairing (GRELL 1962). Moreover, considering the structural nature of compound autosomes, we propose that nonhomologous (distributive) pairing is a property of the centromeric region and suggest that rearrangements involving breaks in this region possibly alter the effectiveness of distributive pairing forces.

COMPOUND autosomes hold an exceptional position within the diversity of chromosomal rearrangements and, as such, they provide a notable genetic tool. Like the reverse metacentric compound-*X*, they arise through the attachment of homologous arms to a common centromere. However, compound autosomes differ from the compound-*X* in their effect on the population—with rare exceptions, organisms bearing compound autosomes are genetically isolated from all other members of the same species that carry standard chromosomes or other forms of chromosomal rearrangements.

In Drosophila, compound autosomes were initially constructed in the laboratory of E. B. LEWIS (RASMUSSEN 1960). In her report, RASMUSSEN described the

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[3] Present address: Department of Zoology, The University of British Columbia, Vancouver, British Columbia V6T 1W5.

formation of attached arms for the major autosomes as the joining of breakpoints on opposite sides of homologous centromeres. Additional theories on compound formation in *Drosophila melanogaster* have been offered (BATEMAN 1968), one of which suggests compounds are products of centromere misdivision. This latter model has historical significance originating with the isochromosomes described by DARLINGTON (1939, 1940). Recent studies, however, strongly support the concept of compound formation as a translocation-type event (LEIGH and SOBELS 1970; HOLM *et al.* manuscript in preparation).

A complementary pair of compound-*2* or compound-*3* autosomes can essentially be viewed as a pair of metacentric heterologs. This change in chromosomal composition produces a distinctive modification in the meiotic properties of the chromosomes involved, thereby providing a means of investigating several areas in genetics for which suitable methods previously had not been available.

By using compound-autosome strains, MCCLOSKEY (1966) demonstrated that sperm, nullosomic for any one arm of the major autosomes, were clearly viable. Following the reports that nonsegregational products are frequently and regularly produced in compound-*3* males (BALDWIN and CHOVNICK 1967; HOLM, DELAND and CHOVNICK 1967), studies were extended to show that sperm could function while carrying only chromosome *4* (LINDSLEY and GRELL 1969). The regular production of diplo- and nullo-sperm by compound-autosome-bearing males also furthered the studies on meiotic mutants affecting oogenesis (SANDLER *et al.* 1968; DAVIS 1969; ROBBINS 1971; BAKER and CARPENTER 1972; HALL 1972) and facilitated investigations on induced and spontaneous nondisjunction and chromosome loss in females (BATEMAN 1968; GAVIN and HOLM 1972; WURGLER, RUCH and GRAF 1972; CLARK and SOBELS 1973).

Since a newly generated compound autosome can be recovered and maintained exclusively in a male line, a homogeneous population of compound autosomes, heterozygous for a given distribution of genetic markers, can be generated to provide, in subsequent generations, females for large-scale half-tetrad analysis (BALDWIN and CHOVNICK 1967). Such studies marked the way to demonstrating gene conversion in higher organisms (CHOVNICK *et al.* 1970; BALLANTYNE and CHOVNICK 1971; CHOVNICK, BALLANTYNE and HOLM 1971).

This paper, which is an elaboration of an earlier report (HOLM, DELAND and CHOVNICK 1967), focuses attention on the meiotic behavior of compound-*3* chromosomes, both in males and in females. The reader is also referred to a report by E. H. GRELL (1970) that describes a number of interesting observations concerning the meiotic behavior of compound seconds in females. The results of the present study suggest that the meiotic behavior of compound thirds in females can be interpreted in terms of the distributive pairing model (GRELL 1962) and support the notion that during spermatogenesis compound-*3* chromosomes assort at random.

MATERIALS AND METHODS

Description of rearranged and compound chromosomes: A general description of genetic markers, compound autosomes, compound-*X* chromosomes and inversion-bearing second chromo-

TABLE 1

Genetic description of the compound autosomes used in this study

Code	Compound-3L autosome
P2	*C(3L)RM,P2,ri/ri*
P5	*C(3L)RM,P5,In(3L)Payne/ve h th*
SH2	*C(3L)RM,SH2,+/+*
SH3	*C(3L)RM,SH3,+/+*
Code	Compound-3R autosome
P2	*C(3R)RM,P2,sr/sr*
P5	*C(3R)RM,P5,sbd²gl e⁸/sbd²gl e⁸*
SC1	*C(3R)RM,SC1, kar ry/kar ry*
SH3	*C(3R)RM,SH3,ry²/ry²*
SH4a	*C(3R)RM,SH4a,In(3R)C,Sb e 1(3)e/ca K-pn*
SH4b	*C(3R)RM,SH4b,ca K-pn/ca K-pn*
SH20	*C(3R)RM,SH20,+/+*
SH21	*C(3R)RM,SH21,+/+*
SK2	*C(3R)RM,SK2,p^p ss e⁸ /cu gl*

somes used in this study can be found in LINDSLEY and GRELL (1968). The inversion-bearing second chromosomes were: *In(2LR)bw^V1*, a chromosome whose centromere is displaced to an acrocentric position, and *In(2LR)SM1,Cy* a multiple-break rearrangement that effectively suppresses crossing over along the entire length of chromosome *2*. Two structurally different compound-*X* chromosomes were employed: *C(1)M3* (originally designated *FMA3* by LEWIS 1958), an acrocentric compound bearing multiple inversions, and *C(1)RM y pn*, a standard reverse-metacentric attached-*X* bearing the markers yellow body (*y*) and prune eyes (*pn*).

The terminology suggested by LINDSLEY and GRELL (1968) has been used to represent the general nature of the compound autosomes. In addition, each compound is given an alphanumeric code. The genetic descriptions and corresponding codes for the compound autosomes used in this study are presented in Table 1. To demonstrate how this terminology is translated, we shall select as a representative strain, *C(3L)RM,P2,ri/ri;C(3R)RM,SH20,+/+*, which is read as follows: compound-3 left, reverse metacentric, Pasadena-2, homozygous radius incompletus and compound-3 right, reverse metacentric, Storrs-H20, homozygous wild type. The codes (e.g., *P2;SH20*) will serve as abbreviations throughout this paper in place of a full description of the compounds. The first letter indicates where the compound was generated, while the second letter and (or) number is specific to an attachment recovered as an independent event.

The formation of compound autosomes: Following the construction of the initial set of compound-3 autosomes (RASMUSSEN 1960), synthesis has been accomplished simply by mating X-ray-treated virgin females, homozygous or heterozygous for a given set of genetic markers on standard third chromosomes (or, when required, heterozygous for paracentric inversions), to males carrying compound-3 chromosomes with markers clearly distinguishable from those introduced by the female parent. Generally, the females are aged three to four days before treatment with 3,500 r of X-irradiation. This is followed by mass matings with compound-3 males in ½-pint creamers containing standard Drosophila medium. Normally one transfer is made after day four, although in our experience the highest recovery of newly generated compounds was made in the first brood.

Surviving progeny, other than those resulting from induced nondisjunction, inherit one compound autosome from their father and a newly generated compound from their mother. Since each newly generated compound is the result of an independent and possibly a unique event, it may possess peculiar properties. Therefore, it is established in a separate stock.

Without a complete understanding of the nature of compound formation and the properties of their centromeric regions we chose to study a number of independently derived compound thirds. The choice of chromosomes for the initial experiments was restricted by the differentially

marked stocks available. Subsequent tests, however, employed newly induced compound lines that demonstrated the best fertility and viability.

The transfer of marked heterologs from standard to compound strains: Although marked heterologs can be introduced simultaneously with the generation of a compound, they can also be introduced as a result of nonhomologous pairing. This is accomplished by generating a population of females with the marked heterolog and heterozygous for inversions in the chromosomes corresponding to the compounds. For example, a compound-*X* is introduced into a compound-*3* strain by mating compound-*X* females structurally heterozygous for chromosome-*3* to compound-*3* males. A sufficient number of *C(1)RM;C(3L);C(3R)* progeny will be recovered from 50 pair matings to establish the new compound line.

General procedures for segregation and hatchability studies: The two parameters that might have a marked effect on meiosis, age and temperature were held within fairly narrow limits. Males and females both were collected within eight hours following eclosion and aged for approximately two days before mating. The crosses for meiotic segregation studies were incubated at 24 ± 0.5°; the hatch studies were conducted in the laboratroy where the temperature remained 25 ± 1°.

In the experiments on meiotic behavior, single females were mated with two or three males of the appropriate genotype in shell vials containing standard cornmeal, molasses, yeast and agar medium, freshly seeded with live yeast. On the third day following mating, the females were transferred to fresh food vials and the males were removed by means of an aspirator. Hereafter, transfers were made every two days for a total of five broods, or eleven days of egg laying. Vials were numbered so that the total number of progeny per family could be used to examine the statistical distribution of exceptional meiotic events.

For the hatchability tests, single females were placed in empty quarter-pint creamers with three males and inverted over 60-mm plastic petri dishes containing standard Drosophila medium to which had been added, to facilitate egg scoring, 4 gms of animal charcoal per litre of food. To firmly support the creamers to the top of the petri dishes, plastic collars adapted from the lids of the dishes were attached to the openings of the bottles.

Every 24 hours the creamers were transferred to petri dishes containing fresh medium. Total eggs were scored immediately after the transfer and hatched eggs were recorded 36 hours later.

RESULTS

Two distinct classes of progeny arise from crosses involving compound-*3* parents. Those receiving either *C(3L)* or *C(3R)* from the female parent and the complementary compound from the male parent are classified as segregational. Progeny inheriting both compounds from the mother (the matroclinous class) or from the father (the patroclinous class) are nonsegregational. The terms "segregational" and "nonsegregational" (as distinct from the terms "disjunctional" and "nondisjunctional" which generally refer to meiotic events between true homologs) have been adopted as operational definitions, not to replace the more conventional terminology, but rather to aid in clearly distinguishing events involving pairs of heterologous compounds from those involving true homologs.

The results entered in Table 2 are from crosses that involved compound-*3*-bearing females structurally homozygous for the standard arrangement of all other chromosomes. The nonsegregational progeny obviously constitute the exceptional class, indicating that nonsegregation within the genetic background represents an infrequent meiotic event. Considering the five crosses independently, we first note that the females used in Experiments 1 and 2 were from the same compound strain. The experiments differ in that males of the second cross

TABLE 2

Percent recovery of nonsegregational progeny from C(3L);C(3R) *females homozygous for the standard* X *and second chromosomes*

Experiment	Parents		Progeny			Homogeneity test
				Nonsegregational		
	Female	Male	Total	Number	Percent	P
1	+/+;*P5;P5*	+/+;*P2;P2*	13,205	74	0.56	.88
2	+/+;*P5;P5*	*SM1*/+;*P2;P2*	4,896	22	0.45	<.01*
3	+/+;*SH2;SH21*	+/+;*P2;SC1*	23,528	140	0.59	.39
4	+/+;*P2;P2*	+/+;*P5;P5*	8,255	322	4.02	<.01
5	+/+;*P2;SK2*	+/+;*SH2;SH3*	4,263	207	4.62	.10

P values in Tables 2, 3 and 4 were obtained using the modified chi square homogeneity test of BRANDT and SNEDECOR (SNEDECOR 1956).

* $P > .05$ with the removal (from the 108 crosses) of one family in which the frequency of nonsegregational progeny was exceptionally higher than the mean.

were structurally heterozygous for chromosome two. The mean frequency of nonsegregation, however, is insignificantly altered by this modification. Females from a second strain (Experiment 3) produced a similarly low frequency of nonsegregational progeny. The female parents involved in Experiments 4 and 5 had in common the *C(3L)P2,ri* chromosome; the males were taken from two different lines. The two results, which show similar frequencies, are consistent with earlier findings (BALDWIN and CHOVNICK 1967) that *C(3L)P2,ri* is associated with a relatively high degree of nonsegregation.

For purposes of comparison, we shall refer to the base level of nonsegregation, for any given pair of compounds, as the spontaneous frequency. This is meant solely as a term of reference to those exceptional events arising in females whose other chromosomes are apparently structurally normal and carry no genetic markers that allow for observations on possible heterolog interaction.

The spontaneous frequency can be viewed in two ways: (1) either these levels of nonsegregation reflect some intrinsic property of the compounds involved, or (2) the frequency is the mean of events involving regular segregation of *C(3L)* and *C(3R)* in the majority of females, plus nonsegregational events occasioned through nonhomologous pairing, with possibly a free *Y* chromosome (GRELL 1970) carried by some fraction of the female population. The two alternatives can be tested by examining the distribution of exceptional events within the population of female parents. If the mean frequency of nonsegregation reflects some property of the compounds, a relatively homogeneous distribution of exceptional events should be found within the sample populations. Even though the average number of exceptional progeny per family is small, because of the high variance in total progeny per family we used the modified chi square test of BRANDT and SNEDECOR (SNEDECOR 1956), without exception, as a measure of homogeneity. We feel this method is quite valid for providing a measure of the degree of dispersion and refer to LEWONTIN and FELSENSTEIN (1965) for a discussion on the validity of this statistical approach.

The last column in Table 2 contains the P values obtained from the homogeneity tests. The results indicate heterogeneous distributions in only two of the five experiments. In Experiment 2, however, the removal of one family, in which there were four exceptional progeny, results in a change of $P < .01$ to $P > .05$. Furthermore, if for Experiment 4 we represent the nonsegregational frequencies by means of a histogram (Figure 1), a skewed but unimodel distribution is obtained. In view of these statistical comparisons we infer that, although some frequencies may be moderately increased by heterozygosity in other chromosomes within the population, the spontaneous level of nonsegregation probably reflects properties intrinsic to the pair of compounds involved.

The four different combinations of compound-*3* chromosomes described above, as well as many others, have been maintained in stocks for as long as eight years. Some are checked periodically and the levels of spontaneous nonsegregation show little change. It is curious, therefore, that at least two different levels of spontaneous nonsegregation should persist. Recent investigations have provided some insight into this problem in revealing a positive correlation between spontaneous nonsegregation and *X*-chromosome nondisjunction. It now would appear that most (but probably not all) nonsegregational events can be attributed to nonhomologous pairing. Moreover, the different levels of nonsegregation appear to be related to interchromosomal effects that are caused by compound autosomes but not necessarily as intrinsic properties of compound formation (HARGER and HOLM 1973, and manuscript in preparation).

The effects of structural heterozygosity in chromosome 2: The effects of introducing structural heterozygosity for chromosome *2* into three different strains of compound-*3* females are recorded in Table 3. Although structural heterozygosity in males is of no obvious consequence (see Experiment 2, Table 2) its influence

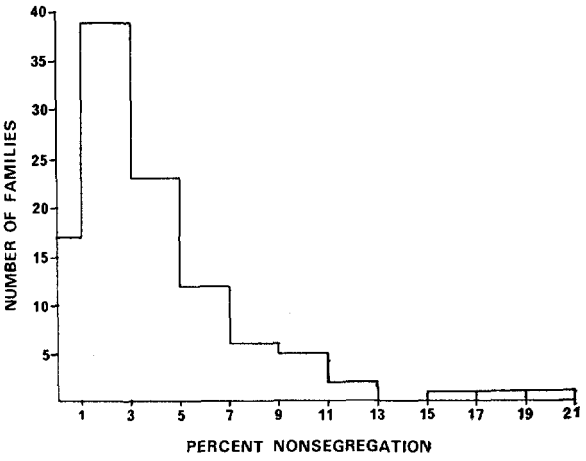

FIGURE 1.—Frequency distribution of nonsegregational progeny recovered (in Experiment 4) from 107 crosses between *C(3L)P2;C(3R)P2* females and *C(3L)P5;C(3R)P5* males. Intervals of percent nonsegregation are recorded on the horizontal axis. The values on the vertical axis indicate the number of families (crosses) contributing to each interval.

TABLE 3

Percent recovery of nonsegregational progeny from C(3L);C(3R) *females heterozygous for chromosome-2 inversions*

Experiment	Parents		Progeny			Homogeneity test
				Nonsegregational		
	Female	Male	Total	Number	Percent	P
6	*SM1/+;P5;P5*	*P2;P2*	13,557	1,785	13.17	.05
7	*SM1/+;P2;P2*	*P5;P5*	5,356	472	8.81	<.01
8	*SM1/+;SH2;SH21*	*P2;SC1*	11,120	1,071	9.63	<.01
9	*SM1/+;SH2;SH21*	*P2;SH4b*	7,644	720	9.42	<.01
10	*SM1/bw^{V1};SH2,SH21*	*P2;SH4a*	4,999	356	7.12	.15
11	*+/bw^{V1};SH2;SH21*	*P2;SH4a*	4,096	89	2.17	<.01**

* *SM1=In(2LR)SM1,Cy; bw^{V1}=In(2LR)bw^{V1}*

** P > .05 with the removal (from 93 crosses) of one family with a frequency of nonsegregational progeny that was exceptionally higher than the mean.

on the distribution of compound thirds during oogenesis is quite apparent. It has been well documented that structural heterozygosity in a single pair of chromosomes leads to little, if any, nondisjunction (see COOPER, ZIMMERING and KRIVSHENKO 1955) and more specifically it was recently demonstrated that the spontaneous level of nondisjunction in females for *Cy,SM1/+* is quite infrequent (GAVIN and HOLM 1972). However, *Cy,SM1* and its standard homolog are either infrequently or never involved in exchange pairing (MACINTYRE and WRIGHT 1966) and therefore, along with the compound autosomes, supposedly form regular members of the distributive pairing pool. As the results recorded in lines 1 through 4 of Table 3 demonstrate, the frequency of nonsegregation is greatly increased by *Cy,SM1/+*. It is interesting to note, however, that the observed levels of increase above the spontaneous frequency are not proportional for the three different combinations of compounds tested. It is important to recognize, therefore, that the observed frequency of nonsegregation does not provide an accurate picture, as only those nonsegregational events in which one second chromosome and either both or neither of the compounds move to the same anaphase pole are recovered in a viable zygote. This problem shall be considered further in a later section of this paper.

In the first four experiments recorded in Table 3, only Experiment 6 provided a homogeneous distribution of exceptional progeny. The remaining three (Experiments 7 to 9), although heterogeneous according to the χ^2 test, gave unimodel distributions with skewing in the direction of increased nonsegregation, similar to the results noted for Experiment 3 (Figure 1). It was considered at this point that such heterogeneity in the recovery of nonsegregational events could arise from the variable presence of *Y* chromosomes.

To select for a population of females free of *Y* chromosomes, we employed the familiar *Y* suppression of variegation (BAKER 1968). The multiple-break inversion, *In(2LR)bw^{V1}* (abbreviated *bw^{V1}*), which carries a dominant brown-variegated eye marker (the expression of which is suppressed by the presence of an

extra *Y* chromosome in males or a single *Y* in females) was introduced into the *C(3L)SH2;C(3R)SH21* strain and balanced over *Cy,SM1*. The expression of the brown-eyed phenotype was greatly enhanced in this strain and very few individuals showed any degree of variegation. Such enhancement of brown-variegated had been demonstrated previously to be associated with heterochromatic deficiencies (MORGAN *et al.* 1941). To verify that an extra *Y* chromosome would suppress the brown-variegated phenotype in this compound strain, bw^{V1}*/Cy,SM1; C(3L);C(3R)* males were crossed to *C(1)RM;C(3L);C(3R)* females that carried at least one free *Y*. The male progeny that inherited bw^{V1} fell into two distinct phenotypic groups. Over 90% expressed distinct, phenotypically brown eyes; the remaining 10% possessed eyes that were clearly variegated. The majority of the female progeny developed brown-variegated eye patterns, quite distinct from the brown-eyed males, while the remainder exhibited eye coloration indistinguishable from wild type. As demonstrated in the following section, the presence of a compound-*X* occasions the greatest degree of observed nonsegregation and, although unverified by other means, the two distinctly different expressions of eye coloration in the bw^{V1} females are interpreted as possible indicators of one and two *Y* chromosomes.

Only those females with phenotypically unmottled brown eyes were selected for Experiments 10 and 11 (Table 3). This resulted in the recovery of a homogeneous distribution of nonsegregational progeny about a mean frequency of 7.1% in Experiment 10, while for Experiment 11, where the nonsegregational frequency is considerably lower, the distribution is homogeneous only if one family, with an exceptionally higher frequency, is removed. This does not result in a significant decrease of the mean nonsegregational frequency. The results of experiments using bw^{V1} suggest, therefore, that heterogeneous distributions in the *Cy, SM1/+*; compound-*3* females were, at least in part, the result of free *Y* chromosomes carried by a small portion of the females in the population.

The above results, along with those presented in the next section, imply that each pair of compounds, in combination with specific heterologous rearrangements, assort in a relatively reproducible distribution. In addition to the homogeneity tests, we note that the frequencies of nonsegregation in consecutive broods of any one experiment, with few exceptions, were statistically constant; and when females from the same strain were used in two separate crosses (compare Experiments 8 and 9) the results were consistent.

*The effect of a compound-*X *chromosome*: Table 4 contains the results from experiments that involved compound-*3* females carrying compound-*X* chromosomes. As noted above, the compound-*X* female population appears to be heterogeneous for one and two *Y*'s. The removal of free *Y* chromosomes was not attempted prior to these experiments, and although preliminary studies using a marked *Y* informed us that compound-*X*;*Y* nonsegregation was also a frequent event in compound-*3* females, the marked *Y* employed so greatly reduced viability that only females carrying normal *Y*'s were used in the present studies. The overall effect of the *Y* chromosome, therefore, can only be implied.

Before considering each cross separately, it should be noted that the frequency

TABLE 4

Percent recovery of nonsegregational progeny from C(3L);C(3R) *females carrying compound-X chromosomes*

Experiment	Parents		Progeny			Homogeneity test
				Nonsegregational		
	Female	Male	Total	Number	Percent	P
12	*C(1)M3;P2;P2*	*+/+;P5;P5*	3,167	644	20.33	.10
13a	*C(1)M3;P5;P5*	*+/+;P2;P2*	7,831	2,087	26.65	.42
13b	*C(1)M3;P5;P5*	*+/+;P2;P2*	4,573	1,196	26.15	.72
14	*C(1)M3;P5;P5*	*SM1/+;P2;P2*	8,030	2,131	26.54	<.01*
15	*C(1)M3;SH2;SH21*	*+/+;P2;SC1*	5,547	1,446	26.07	<.01*
16	*C(1)RM;SH2;SH21*	*+/+;P2;SC1*	4,987	1,359	27.25	.05

* The removal (from 196 crosses) of one family in Experiment 14 and (from 178 crosses) of four families in Experiment 15 provides homogeneity ($P \geqslant .05$) without significantly decreasing the mean frequency of nonsegregation (see text).

distributions, with only minor adjustments in two of the experiments (as noted in Table 4), are quite homogeneous, even though heterogeneity in numbers of free *Y*'s is suspected. The exceptional females that contributed to the high chi square values were few (one in Experiment 14 and four in Experiment 15), and in each case they produced greater than 50% nonsegregational progeny.

The mean frequencies of nonsegregation (Table 4) are essentially the same in four of the five experiments. The exception (Experiment 12) involved the *C(3L)P2,ri* chromosome which, interestingly, was associated with the highest recovery of nonsegregational progeny in the absence of known heterologous rearrangements. The results for Experiment 13 are divided into two groups. Group one (Experiment 13a) represents the total progeny recovered in the first five broods. On day 12, the females were remated with young (2–3-day old) males, which were removed after the second day (day 14). The females were then transferred through three further two-day broods for a total of eight additional days of egg laying (Experiment 13b). As the results in Table 4 indicate, there was no change in the mean nonsegregational frequency with aging of the females, and the distribution remained homogeneous. Consistent with the results presented in Table 2, structural heterozygosity of chromosome-*2* in males (Experiment 14) exercises no obvious influence on the distribution of compound autosomes during spermatogenesis.

The last two experiments concern the meiotic distribution of the same pair of compound autosomes in combination with two structurally different compound-*X* chromosomes. One of these compounds [*C(1)M3*] is a multiple-break rearranged compound whose attached arms cannot freely engage in exchange pairing, whereas the other [*C(1)RM*] is a reverse metacentric in which crossing over is apparently unimpaired. The general influence of these two different compounds (Experiments 15 and 16), based on a comparison of the two mean frequencies of compound-*3* nonsegregation, is insignificantly different.

The meiotic distribution of compound-3 chromosomes in males: In the results

cited above, only alterations to the female genome produced significant changes in the recovery of nonsegregational events. Earlier results (BALDWIN and CHOVNICK 1967; HOLM, DELAND and CHOVNICK 1967) were interpreted to indicate that nonsegregation of compound-*3* autosomes in males occurs regularly and at a high frequency. The results of the present experiments unquestionably support this interpretation. SCRIBA (1967, 1969), who carried out egg-hatching studies on compound-*2* and compound-*3* strains, found that approximately 80% of the eggs laid by compound-bearing females showed embryonic lethality. He interpreted these results to indicate random assortment during gametogenesis in both sexes. This is obviously not the case. However, as demonstrated by the theoretical model presented in Figure 2, random assortment of compound autosomes, in males only, will give the same predictable result.

Supported by the conclusion of SCRIBA (1969) that all aneuploid states of compound-*3* autosomes lead to embryonic lethality, and predicting that each of the four possible classes of sperm produced by compound-*3* males occur with equal frequency (0.25), we find, according to the equation in Figure 2, that the expected egg hatch is 25% for all values of x (the frequency of segregation in females). In addition, we must assume that all other chromosomes disjoin regularly, and if nondisjunction occurs, the percent hatch will decrease accordingly. Table 5 gives the results of experiments designed to test these predictions.

Each test for egg lethality involved 25 to 30 females individually placed with two males in special egg-scoring containers (see METHODS AND MATERIALS). The parents were transferred every 24 hours to new containers for a total of five or six days of egg laying. To eliminate the possible inclusion of unfertilized eggs, scoring was initiated on that day following the first observed hatch. The data in Table 5 include in addition to the total number of eggs recorded during the egg-laying period, the mean percentages of eggs hatched and the 95% confidence intervals calculated from the arcsin transforms of individual results (ROHLF and SOKAL 1969).

With the exception of Experiments C3–3 and C3–4, in which the females were structurally heterozygous for chromosome-*2*, the results are in good agreement

Meiotic Assortment of Compound-3 Chromosomes

	Female Gametes			Male Gametes		Frequency of viable eggs
	Chromosome	Frequency		Chromosome	Frequency	
S	C(3L)	x/2	X	C(3R)′	0·25	0·125x
	C(3R)	x/2	X	C(3L)′	0·25	0·125x
N	C(3L);C(3R)	(1-x)/2	X	0	0·25	0·125(1-x)
	0	(1-x)/2	X	C(3L)′;C(3R)′	0·25	0·125(1-x)
				Total viable zygotes (expected hatch)		0·25

FIGURE 2.—Expected frequency of egg hatch based on random assortment of *C(3L)* and *C(3R)* chromosomes in males. S = segregational meiosis; N = nonsegregational meiosis.

TABLE 5

Summary of studies on the percent hatch of eggs recovered from the indicated crosses

Experiment	Parents		Total eggs	Mean percent† hatch	95% C.I.
	Female	Male			
A	+/+*	+/+	4,321	90.5	88.5–92.5
B	+/+	+/+	3,730	90.2	87.2–92.9
C3–1	*P2;P2*	*P2;P2*	4,288	22.4	20.7–24.2
C3–2	*P2;P2*	*SM1/+;P2;P2*	4,584	21.5	19.6–23.4
C3–3	*SM1/+;P2;P2*	*P2;P2*	4,722	13.4	12.3–14.6
C3–4	*SM1/+;P5;P5*	*P5;P5*	7,167	17.0	16.0–18.1
C3–5	*P5;P5*	*SM1/+;P5;P5*	7,416	22.3	21.4–23.3
C3–6	*SH2;SH21*	*P5;P5*	3,123	20.7	19.1–22.3
C3–7	*SH2;SH21*	*SH2;SH21*	6,429	25.8	23.4–28.3
C3–8	*SH2;SH21*	*P2;SC1*	3,619	25.3	23.0–27.7
C3–9	*SH3;SH20*	*SH3;SH20*	7,413	23.3	21.8–24.8

* +/+ = Oregon-R wild type.
The mean percent hatch and the 95% confidence intervals were determined by using the arcsin transformation values of individual results.

with the model. As a general control, the percent hatch was measured for an Oregon-R strain from which a number of wild-type compound lines had been generated. The other compound lines, however, were derived from various mutant-bearing stocks at Storrs and at Pasadena. Even with compounds generated from the Oregon-R strain, the alterations to the chromosomes as a result of compound formation, and the possible effects of radiation on the genetic background, in general, must be considered. It is interesting, nevertheless, to note that in a number of experiments, adjusting the mean frequency of hatch of the compound strain for embryonic lethality observed in the standard cross produces a mean value that closely approximates the predicted 25%.

Regarding the effects of structural heterozygosity, we find that in Experiments C3–2 and C3–5, where the males were heterozygous for *Cy,SM1*, the percent hatch falls well within the expected limits. In clear contrast to these results, structural heterozygosity for chromosome *two* in females (Experiments C3–3 and C3–4) produces a significant increase in egg lethality. In view of the results recorded in Table 3, this increase in egg lethality is not unexpected. As previously suggested, if increased nonsegregation of compounds reflects nonhomologous pairing with nonexchange second chromosomes, the observable events will be only those in which both compounds and one second chromosome are directed to the same pole. It is probable, therefore, that an equal or even greater proportion of nonhomologous pairing events will result in nondisjunction of the structurally heterozygous seconds. We note in Table 5 (comparing Experiments C3–2 and C3–3) that a 38% decrease in hatch occurs when *Cy,SM1* is introduced into the *C(3L)P2;C(3R)P2* females. In contrast, *Cy,SM1* in *C(3L)P5;C(3R)P5* females (compare Experiment C3–4 and C3–5) reduces hatch by only 24%. When the percent hatch is compared with the frequency of nonsegregation (Table 3) for

the corresponding females, we realize that the highest recovery of hatched eggs came from those females that produced the greatest proportion of exceptional progeny. Therefore, it is apparent from these observations that the total effect on nonhomologous interactions, where only major autosomes are involved, cannot be predicted solely by measuring the frequency of nonsegregation. Furthermore, it might be inferred from these observations that the distribution of nonexchange and compound autosomes might be governed to some degree by either properties of preferential secondary pairing or preferential segregation. This argument shall be pursued further in the following section.

The nonrandom assortment of nonhomologous chromosomes: That disruption of regular segregation of compound thirds arises as an outcome of interaction with the structurally heterozygous seconds seems quite apparent from the preceding results. When all four major autosomes are involved in nonexchange distribution, a 3:1 segregation commonly occurs; and since the structurally heterozygous second chromosomes were distinctly marked, it is possible to compare the distribution of nonsegregational compounds with either of the heterologs. For those crosses involving a compound-*X*, the presence of unmarked *Y* chromosomes restricts our attention to only three of the four (and possibly five) chromosomes involved in nonhomolog assortment. Nonetheless, the results lead to the implication that preferential pairing followed by segregation is involved in the distribution of these chromosomes.

In Table 6 we have listed, for those experiments that involved females either structurally heterozygous for chromosome-*2* or carrying a compound-*X*, the frequency distribution of the *marked heterolog* in the disomic-*C(3)* and nullosomic-*C(3)* eggs. The experiments are grouped according to the heterolog

TABLE 6

*The distribution of structurally heterozygous second and compound-*X *chromosomes in the disomic-*C(3) *and nullosomic-*C(3) *eggs*

Experiment*	Female parent	Marked heterolog	Distribution of heterolog in nonsegregational eggs: Disomic-*C(3)* Number	Disomic-*C(3)* Percent	Nullosomic-*C(3)* Number	Nullosomic-*C(3)* Percent
6	*SM1/+;P5;P5*	*SM1,Cy*	621	34.80	1,164	65.20
7	*SM1/;P2;P2*	*SM1,Cy*	166	35.17	306	64.83
8	*SM1/+;SH2;SH21*	*SM1,Cy*	596	55.65	475	44.35
9	*SM1/+;SH2;SH21*	*SM1,Cy*	398	55.28	322	44.72
10	*SM1/bw^{V1};SH2;SH21*	*SM1,Cy*	72	20.22	284	79.78
11	*+/bw^{V1};SH2;SH21*	*bw^{V1}*	60	67.42	29	32.58
12	*C(1)M3;P2;P2*	*C(1)M3*	274	42.55	370	57.45
13a	*C(1)M3;P5;P5*	*C(1)M3*	628	30.09	1,459	69.91
13b	*C(1)M3;P5;P5*	*C(1)M3*	345	28.85	851	71.15
14	*C(1)M3;P5;P5*	*C(1)M3*	660	30.97	1,471	69.03
15	*C(1)M3;SH2;SH21*	*C(1)M3*	431	29.81	1,015	70.19
16	*C(1)RM;SH2;SH21*	*C(1)RM*	267	19.65	1,092	80.35

* Experiment numbers are provided for reference to Tables 3 and 4.

involved and, for reference to earlier tables, the corresponding experimental numbers are included.

The first four entries in Table 6 describe the distribution of *Cy,SM1* in the nonsegregational classes of compound autosomes. The distribution of heterologs departs significantly from randomness and there is an inconsistency in the pattern, which conceivably depends on the combination of chromosomes involved. It is interesting to observe, however, that while the same distribution does not arise for every combination of compounds, the results appear to be highly reproducible for any given pair. For example, note the constant ratio, in subsequent broods, for the nonsegregational progeny in Experiment 10 (compare columns 1 and 4 with columns 2 and 3 in the nonsegregational classes recorded in Table 7). Note further the agreement between repeat experiments involving *Cy,SM1; C(3L)SH2;SH21* females (Experiments 8 and 9, Table 6).

By far the most convincing example of nonrandom assortment was demonstrated in Experiment 10 (line 5, Table 6). Eighty percent of the progeny

TABLE 7

Brood analysis on the distribution of chromosomes recovered in progeny from the cross SM1,Cy/bw^{V1};C(3L)SH2,+;C(3R)SH21,+ *females* × C(3L)P2,ri;C(3R)SH4a *males in Experiment 10*

	Segregational progeny						
Brood	*C(3L)SH2;C(3R)SH4a* *bw^{V1}*	*Cy*	*C(3L)P2;C(3R)SH21* *bw^{V1}*	*Cy*	Total	χ^2	P
1	276	291	316	296	1,179	1.95	
2	316	312	295	310	1,233	4.16	
3	260	258	310	318	1,146	4.48	
4	181	154	180	181	696	1.17	
5	111	80	102	96	389	4.11	
Total	1,144	1,095	1,203	1,201	4,643	15.86	0.2
	Nonsegregational progeny						
	Matroclinous		Patroclinous				
Brood	*C(3L)SH2;C(3R)SH21* *bw^{V1}*	*Cy*	*C(3L)P2;C(3R)SH4a* *bw^{V1}*	*Cy*	Total	χ^2	P
1	41	8	5	36	90	2.08	
2	34	10	12	30	86	2.41	
3	40	12	10	33	95	.53	
4	22	5	3	17	47	.58	
5	16	4	3	15	38	.17	
Total	153	39	33	131	356	5.77	0.9
	Total progeny						
	Segregational		Nonsegregational				
1	1,179		90		1,269	.00	
2	1,233		86		1,319	.72	
3	1,146		95		1,241	.54	
4	696		47		743	.71	
5	389		38		427	2.04	
Total	4,643		356		4,999	4.01	0.4

recovered from this cross represent meiotic events in which both compounds segregated from the *Cy,SM1* chromosome. This same pair of compounds was carried by the females used in Experiments 8 and 9, where nonsegregational compounds segregated from *Cy,SM1* in only 45% of the exceptional events. Therefore, by replacing the standard second with *In(2LR)bw*^{V1}, a dramatic shift in the pattern of nonhomolog assortment is realized. A similar distribution is recognized, but to a lesser extent, for the combination +/*bw*^{V1} in Experiment 11 (line 6, Table 6). That differential viability does not make a significant contribution to this apparent preferential assortment is demonstrated by the equal recovery of the two heterologs both in the segregational and nonsegregational classes (note distribution of *bw*^{V1} and *Cy,SM1* in Table 7).

The remaining entries in Table 6 describe the distribution of heterologs in those experiments involving compound-*X* females. It would appear from this Table that, with the exception of Experiment 12 (entry 7), a highly nonrandom distribution occurs between the compound autosomes and the compound-*X*. In the absence of genetically marked *Y* chromosomes, however, the observed distributions can be quite misleading. In fact, if nonsegregation of the autosomes is a function of random nonhomologous pairings involving either the compound-*X* or a free *Y*, then whether we propose a trivalent or a bivalent pairing model, the recovery of compound-*X* and compound autosomes in separate gametes will be in the order of 75%. Moreover, compound-*X*:compound autosome segregation that is significantly less than 75% indicates greater nonhomologous pairing with the *Y*—a possible explanation for all experiments involving *C(1)M3*, in particular Experiment 12 where the compound-*X* may be assorting almost at random. In fact, only in Experiment 16 (Table 6), where the females carried a reverse-metacentric compound-*X*, does the distribution of chromosomes in the nonsegregational gametes imply preferential segregation from the compound-*X*. The possible role of structural conformation of the chromosomes in preferential nonhomologous pairing and distributions will be considered in the DISCUSSION.

DISCUSSION

Features of compound autosome structure and meiotic behavior pertinent to this discussion are the following: (1) With the possible exception of duplications for proximal heterochromatic segments arising as a consequence of compound autosome formation, compound-*3R* and compound-*3L* are nonhomologous chromosomes. (2) In the absence of heterologous rearrangements, compound autosomes, during oogenesis, segregate with a relatively high frequency. However, (3) in the presence of a compound-*X* and a free *Y* or a structurally heterozygous pair of second chromosomes, a striking decrease in the frequency of segregational events is witnessed. (4) The nonrandom distribution of chromosomes in the products of nonsegregational events, observed in a number of crosses, implies either preferential association or assortment of nonhomologs. Of special interest in this regard is the possible influence of centromere position on chromosome interactions.

In addition to the above features on compound autosome behavior during oogenesis, the results of this study are interpreted as demonstrating that *C(3L)* and *C(3R)* assort independently during spermatogenesis. If, in addition, we can assume that fertilization by any one of the four different sperm products is equally probable, then the distribution of compounds in the progeny will provide, in the absence of chromosome-*2* nondisjunction, a direct measure of nonsegregational frequencies during oogenesis. Since nonsegregational events involving simultaneous nondisjunction of the structurally heterozygous second chromosomes will lead to lethality, which is evident from the large increase in egg lethals, the increased recovery of chromosome-*3* nonsegregational products serves only to demonstrate further that nonhomologous interactions are involved. Moreover, comparing relative nonsegregational frequencies that arise from interactions with structurally heterozygous seconds cannot be interpreted as meaningful since nonrandom distributions of different nonhomologous chromosomes may strongly influence the production of recoverable, nonsegregational products.

Results from numerous studies in the past (for example see COOPER, ZIMMERING and KRIVSHENKO 1955; OKSALA 1958; GRELL 1959; FORBES 1960; GRELL and GRELL 1960) have been consistent with the notion that nondisjunction of non-crossover chromosomes, as well as compound-*X* chromosomes (RAMEL 1962; GRELL 1963), invariably arises from apparent nonhomologous pairing events. The observed meiotic distribution of compound autosomes is in keeping with this concept. Furthermore, since pairs of compound autosomes are essentially heterologs, nonhomologous pairing interactions, in general terms, can be viewed as the exclusive means by which their distribution is determined.

A more specific frame of reference in which the meiotic behavior of compound autosomes can be described is provided by the distributive pairing model (GRELL 1962). According to this model, during meiosis in female Drosophila, segregation of chromosomes is regulated by two successive pairing events. The first event, which involves true synapsis, is described as exchange pairing. Subsequent to exchange, all chromosomes that failed to undergo crossing over with an independent homolog enter a secondary pairing stage called a "distributive pairing pool" or "distributive pairing phase" (GRELL 1969). When only two chromosomes enter the secondary phase, independent of homology, they normally disjoin when the nucleus divides. If more than two chromosomes, unrestricted by exchange pairing, enter the distributive phase, pairing is described as competitive and may result in the nondisjunction of homologs. Competitive distributive pairing is not viewed, however, as a random association of homologous and nonhomologous chromosomes, but rather as a function of size (GRELL 1964). The size rule, deduced primarily from studies on chromosome *four* and *X* duplications, requires that the segregation frequency of small heterologs approaches 100% when the ratio of their physical lengths approaches unity (GRELL 1964). Recent studies on compound fourths (MOORE and GRELL 1972) have added support to the size rule, and, in addition, have indicated that total chromosome length, rather than arm length, determines distributive pairing recognition.

Let us consider the meiotic distribution of compound autosomes, in operational terms, as a function of distributive pairing forces. Consider first, that, as a regular property of all chromosomes, distributive pairing forces operate independently of exchange pairing mechanisms. Secondly, in view of regular crossing over between isosequential arms of compound chromosomes, consider distributive pairing as a property of the centromere or the chromosomal segments immediately flanking the centromere. When independent homologs of standard sequence synapse, their centromeric regions, owing to exchange, are "locked in" to the pairing complex. As a consequence, secondary (distributive) pairing forces within the centromeric regions will be restricted to the homologous pair. In contrast, crossing over between the dependent homologous arms of a compound chromosome will not result in locking homologous centromeres into a bivalent formation; therefore, secondary or distributive pairing forces will be nonrestrictive. When other than the fourth chromosomes, the compound thirds represent the only members of the genome for which centromeric pairing has not been limited by exchange, distributive pairing between the two compounds follows as a regular event. However, when nonrestrictive distributive pairing alternatives are present (e.g., a compound-*X*, a free *Y* or a pair of structurally heterozygous autosomes), distributive pairing is not limited, and pairings involving the various alternatives result in the production of gametes nonsegregational for the compound autosomes.

While the distributive pairing model predicts that meiotic assortment, of compound autosomes as well as the smaller fourth chromosomes, is solely under the influence of distributive pairing forces, major size differences between the two will serve as a barrier to competitive nonhomologous pairing interactions. In view of the low frequency of nonsegregation in the absence of heterologous rearrangements, we acknowledge that chromosome *four* is not an apparent effective pairing alternative for the compound thirds. Nevertheless, while size differences may serve to prevent, under competitive conditions, pairing interactions between chromosomes that are grossly dissimilar in size—a condition that possibly arose through natural selection in response to structural polymorphism—we do not suggest that similar restrictions influence nonhomologous pairing interactions involving large chromosomes only.

Nonrandom distribution of large chromosomes from an apparent competitive distributive pairing phase was most notable where one of the chromosomes was acrocentric as the result of structural rearrangements. While at present it is difficult to envision centromeric displacement *per se* as altering the competitive nature of the distributive pairing forces, it is of interest to note that the acrocentrics arose through inversions with breakpoints that disrupted the continuity of the centromeric heterochromatin. If, as suggested previously, distributive pairing forces are properties of the centromeric region, discontinuities within this region conceivably could weaken the competitive pairing ability of such chromosomes. Although in the present study the crosses involving the acrocentric second, *In(2LR)bw*^{V1}, (and perchance the crosses involving the compound-*X*, *C(1)M3*), served only to direct attention to this possibility, recent observations by CAVERS and HOLM (1973) provide support in favor of this concept. The latter studies

compared the effect of a *C(1)RM* chromosome with the *C(1)DX* (an acrocentric compound-*X* deficient for segments of proximal heterochromatin that probably include the nucleolus organizer; see LINDSLEY and GRELL (1968) for a full description), in the presence of a free *Y*, on the meiotic distribution of structurally heterozygous autosomes. The *C(1)RM* chromosome generally segregated from the nondisjoining pair of autosomes, whereas the *C(1)DX* chromosome appeared to assort at random, with the *Y* chromosome providing the only competitive pairing alternative.

There is one additional point regarding our view that secondary pairing forces are properties of the centromeric region. As an alternative to the distributive pairing model, NOVITSKI (1964) proposed that pairing initiates prior to exchange through chromocentral association of all chromosomes. While our findings agree with the general concept of chromocentral pairing, we cannot escape the argument that forces responsible for the nonrandom assortment of noncrossover and compound chromosomes act secondarily to exchange. Consequently, we consider features of both models pertinent to the interpretation of our results and suggest, therefore, that neither model should be considered completely independent of the other.

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Corresponding editor: B. H. JUDD