EVIDENCE FOR THE SINGLE PHASE PAIRING THEORY OF MEIOSIS[1]

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Manuscript received January 7, 1974

ABSTRACT

The segregation pattern of an attached *X* chromosome with several Y-autosome translocations conflicts with the expectations based on the distributive pairing hypothesis because the chromosomes segregating from the translocation configuration include both exchange and non-exchange chromosomes. The results of the second experiment involving three compound chromosomes go even further; they suggest that the essential association which determines the segregation of nonhomologous elements is in fact set up prior to the time of crossing over.

OF the numerous theories that have been put forward to account for the unusual phenomena presented by meiosis in Drosophila females, none is more imaginative or has received more attention than the "distributive pairing" hypothesis, according to which two distinctly different processes occur, the first involving exchange pairing between homologs, during which crossing over may or may not take place, and a second pairing process (distributive pairing), involving homologous chromosomes which have not undergone exchange, compound chromosomes like attached-*X*, whether or not they have undergone exchange, small chromosomes which regularly do not exchange, etc. These two pairing processes have been postulated as distinctly different phenomena separated in time during the first meiotic division, the first occurring prior to the time of ordinary crossing over, that is, at interphase or early prophase, and the latter subsequently, possibly as late as the succeeding anaphase. The rules governing these two pairing processes are considered to be different, the first depending primarily on homology and the second on other characteristics, such as physical length. This hypothesis has been advanced in a large series of experiments described in a number of publications, the references to which can be found in GRELL (1970).

It has been pointed out that many of the phenomena which can be attributed to a pairing phase subsequent to exchange can equally well be interpreted as the consequence of a chromocentral configuration, hypothesized to occur during interphase and prophase of the first meiotic division. This is not a brand new idea since the existence of a chromocenter has been postulated by a number of workers on various genetic grounds (SCHULTZ and REDFIELD 1951; OKSALA 1958; NOVITSKI 1964; REDFIELD 1964; MERRIAM 1967). According to this idea, the initial "pairing" process occurs prior to interphase of meiosis when the chromo-

[1] Supported by a research grant from the National Institutes of Health, GM18678.

somes, having completed the last premeiotic mitosis, have their centromere regions apposed, with a subsequent formation of the chromocenter. This, then, brings homologs into proximity so that they may exchange segments, after which the remaining chromosomes find themselves in a configuration which will determine their subsequent segregation pattern.

In principle, the distinction between these two alternatives is not difficult to make. If one were able to follow the course of meiosis visually in the female of Drosophila, one would expect to see, according to the first hypothesis, a distinctly new pairing event occurring subsequent to exchange, probably (although not necessarily) involving condensed chromosomes. According to the second, the association of chromosomes, involving non-homologs as well as homologs, would occur prior to the termination of prophase of the first division, with no substantial pairing phenomenon subsequent to the time of exchange of homologs.

Making an experimental distinction between these two possibilities is not simple—for, by and large, genetic data will be as easily interpreted on one basis as on the other. In fact, the majority of published data which appear to support the distributive pairing hypothesis do little more than describe the way in which selected chromosomes, usually non-homologs or compounds, are found in the gametes. Possibly some confusion arises from the ambiguous application of the word "pairing", for not only is it used to refer to the process by which chromosomes come together (conjunction), the state of homologous association (synapsis) and the manner of separation (disjunction), but it is also commonly used to designate an association of more than two members, despite simple etymological considerations. Furthermore, logically indisputable conclusions with respect to the fundamental cytological nature of all these aspects of "pairing" are to be achieved by results from genetic tests, where our observations are clearly limited to the distributions of chromosomes in the end product, the gamete. Even when direct observations in this cytologically difficult material appear to support one hypothesis over another, as does the presence of a chromocenter reported by DÄVRING and SUNNER (1973) in Drosophila oocytes, the final resolution of the question may depend on critical genetic experiments. In fact, virtually all our knowledge of female meiosis in Drosophila comes from genetic experiments rather than from cytological observations.

Indeed, some workers in the field feel that it may be fundamentally impossible to distinguish between these hypotheses. In this paper we describe an experimental set-up indicating that there is no distinction between the chromosomes that are undergoing "exchange pairing" and those that are undergoing "distributive pairing" in that they can participate in the same pairing configuration. Subsequently we describe an experiment which strongly suggests that the pattern of segregation of chromosomes in the so-called "distributive pool" is determined *prior* to the time of exchange, and not after.

TRANSLOCATION EXPERIMENT

EXPERIMENTAL PROCEDURES

Five *Y*-autosome translocations (from the translocations derived by LINDSLEY and SANDLER *et al.* 1972) were obtained from the Pasadena stock center. These

translocations were chosen because males carrying them are fertile without a free *Y* chromosome, and secondly, because they have breaks near the centromeres of the autosomes, allowing for maximum opportunity for crossing over in the individual chromosome arms. Two were translocations with the third chromosome and three with the second. The argument behind this experiment is as follows: If we select those cases where a crossover has occurred in both the left and the right arms of the autosome (see Figure 1) then these chromosomes, according to the d.p.h. (distributive pairing hypothesis) are considered to be in the exchange pool and, consequently, the attached-*X* which, being a compound, must be a member of the so-called distributive pool, will go at random at meiosis with respect to segregation of the translocation complex. Consequently, one should find equal numbers of male and female double crossover progeny since the compound *X* must (according to the d.p.h.) segregate randomly from the complex. On the other hand, the chromocentral theory suggests an alternative result, that even when the homologous regions of the translocation are involved in exchange, association of the attached-*X* with the *Y* involved in the translocation may give rise to segregation such that the attached-*X* will be found more

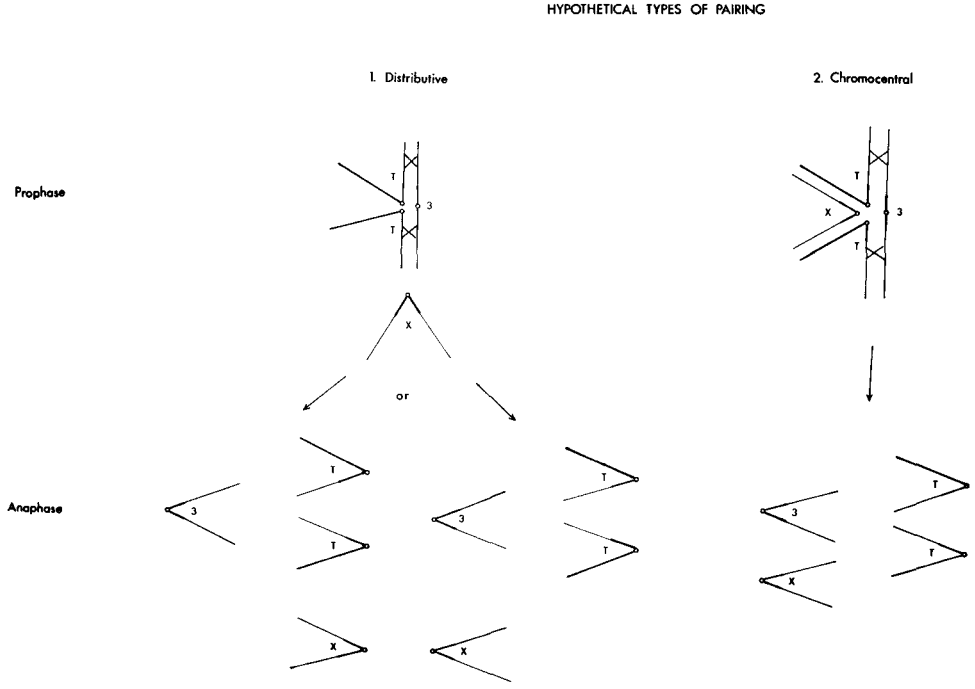

FIGURE 1.—Schematic representation of the two types of pairing: In the first, the "distributive", the *Y-3* translocation (T) is paired with the normal third and has undergone a pericentric double exchange; the compound *X* chromosome, as a mandatory member of the distributive group, must segregate at random from the translocation complex giving rise to four types of gametes expected with equal frequencies. In the second, the chromocentral or single pairing phase model, on occasion the compound will pair with the translocation, which may then segregate "alternately" to give an increment of two of the four types, predicting a departure from a 1:1:1:1 ratio of an excess of translocation males and non-translocation females.

frequently in gametes not carrying the translocation and *vice versa*. The expectation of equality of all four products of segregation as shown in Figure 1, demanded by the d.p.h., is replaced by another possibility, that an excess of the two segregational types shown under "Chromocentral" in Figure 1 will produce a deviation predictable in direction, although not in magnitude, since the frequency of "alternate" segregations will depend upon the specific translocation.

RESULTS

The detailed crossover results for two of the translocations tested are shown in Tables 1 and 2. Our interest is in those classes where a crossover has occurred

TABLE 1

Progeny of y/0;ru 1 h 2 st 3 . 4 p^p 5 ss 6 e/TY-3, A95, y^+ B ♀ ♀ × ru h st p^p ss e ♂ ♂
F_1 carrying *B* not classifiable for *ru*; 1,4, 1,5 and 1,6 therefore not counted as pericentric multiples.

Crossover region	Segregation pattern: $\bar{X}\bar{X} \longleftrightarrow TY\text{-}3$ y^2♀	$\bar{X}\bar{X} \longleftrightarrow TY\text{-}3$ *B*♂	$\overline{XX}, TY\text{-}3 \longleftrightarrow 0$ *B*♀	$\overline{XX}, TY\text{-}3 \longleftrightarrow 0$ non *B*♂	Subtotals	Totals
Non-crossover	275	510	211	71	...	1067
Singles 1	152	—	—	42	194	
2	94	156	43	32	325	
3	14	26	6	6	52	
4	0	1	0	0	1	
5	34	42	8	16	100	
6	60	117	22	26	225	
Subtotals	354	342	79	122		897
Doubles 1,2	29	—	—	10	39	
1,3	5	—	—	0	5	
1,4	1	—	—	0	1	
1,5	12	—	—	9	21	
1,6	30	—	—	14	44	
2,3	1	1	1	0	3	
2,5*	23	15	2	8	48	
2,6*	22	33	7	11	73	
3,4*	2	0	0	0	2	
3,5*	2	2	0	2	6	
3,6*	1	6	0	1	8	
5,6	0	0	0	1	1	
Subtotals	128	57	10	56		251
Triples 1,2,5*	3	0	0	0	3	
1,2,6*	3	0	0	1	4	
1,3,6*	1	0	0	0	1	
2,3,6*	0	1	1	0	2	
2,5,6*	0	1	1	1	3	
3,4,6*	5	0	0	0	5	
Subtotals	12	2	2	2		18
Grand totals	769	911	302	251		2233

TABLE 2

Progeny of y/0;ru 1 h 2 st 3 . 4 p^p 5 ss 6 e/TY-3, G101, y^+ ♀ × ru h st p^p ss e ♂

	Segregation pattern					
	$\overline{XX} \longleftrightarrow TY\text{-}3$		$XX, TY\text{-}3 \longleftrightarrow 0$			
Crossover region	y^+♀	$TY\text{-}3$♂	$TY\text{-}3$♀	non B, non $TY\text{-}3$♂	Subtotals	Totals
Non-crossovers	81	94	48	30		253
Singles 1	25	36	17	13	91	
2	27	37	22	14	100	
3	4	3	2	6	15	
4	2	1	0	1	4	
5	25	20	5	14	64	
6	17	29	9	30	85	
Subtotals	100	126	55	78		359
Doubles 1,2	4	6	5	2	17	
1,3	2	0	2	0	4	
1,4*	2	0	0	0	2	
1,5*	6	9	8	3	26	
1,6*	11	15	6	6	38	
2,3	3	0	1	0	4	
2,4*	1	0	0	0	1	
2,5*	13	8	4	8	33	
2,6*	8	5	3	2	18	
3,5*	7	0	2	0	9	
3,6*	1	0	1	0	2	
4,5	1	0	0	0	1	
5,6	1	0	1	1	3	
Subtotals	60	43	33	22		158
Triples 1,2,5*	1	0	0	1	2	
1,2,6*	3	1	2	3	9	
1,3,5*	1	0	1	0	2	
1,4,5*	1	0	0	0	1	
1,4,6*	1	0	0	0	1	
1,5,6*	1	0	1	1	3	
2,3,6*	1	0	0	1	2	
3,5,6*	0	0	0	1	1	
Subtotals	9	1	4	7		21
Quadruples 1,2,5,6*	2	0	0	0	2	
1,2,3,5*	0	0	0	1	1	
1,3,5,6*	2	1	0	1	4	
Subtotals	2	1	0	2		7
Grand totals	254	265	140	139		798

in each of the arms of the autosome, in order to specify that all the relevant arms have been involved in exchange. These classes, which may be termed "pericentric multiples", are designed with asterisks in the tables.

Of the five translocations tested, the two *Y-3* translocations were done most exhaustively, with all progeny being classified. The presence of both y^+ and *Bar*

TABLE 3

Summary of pericentric multiples from the two experiments detailed in Tables 1 and 2, and three smaller experiments involving translocations between the Y *and the second chromosome*

	$T \longleftrightarrow XX$		$T, XX \longleftrightarrow 0$	
TY-3, A95	58	62	11	24
TY-3, G101	44	60	28	35
TY-2, J30	3	4	0	0
TY-2, G113	*	60	35	*
TY-2, H118	10	5	2	9

* In the case of TY-2, G113, the heterozygous females were mated to $y+$ males, making it impossible to distinguish between the two classes of males.

on the A-95 translocation made it possible to classify all progeny unambiguously, except for the mutant *ru*, which could not be classified in the *Bar* classes. Translocation G-101 lacked *Bar*, and the male progeny, which had a normal allele of yellow on the *X* chromosome, were classified as translocation-bearing, or not, depending on the presence of the well-known "Hairy wing" effect, evidenced when an extra *X*-chromosome tip, from the translocation, was also present. Three *Y-2* translocations were tested less extensively, in smaller experiments, to determine whether they might show the same deviations from the 1:1:1:1 ratio as the *Y-3* translocations did. Only the pericentric multiples were counted, and the totals are entered in Table 3. From that table it can be seen that segregation of the compound *X* from the translocation, where all elements of the translocation are involved in exchanges, is inconsistent with the distributive pairing hypothesis, since the compound *X* chromosome, which should segregate randomly with respect to the translocation according to that hypothesis, instead segregates preferentially from the *Y* translocation.

ATTACHED AUTOSOME EXPERIMENT

EXPERIMENTAL PROCEDURES

Although the preceding experiment shows that crossover and non-crossover chromosomes do not behave independently at meiosis, but may be involved simultaneously in a single configuration determining their mode of segregation, thereby providing a powerful argument against the d.p.h., it does not provide any information as to whether this association of non-homologous chromosomes existed either prior to, or at, the time of genetic exchange. Such evidence, if it could be obtained, would support a chromocentral model as the alternative to the d.p.h., rather than some third model.

A case for the chromocentral model can be made on the basis of a *Y*-chromosome effect on crossing over, as shown by the workers referred to earlier and, in particular, by MERRIAM (1968). The interpretation of this effect, on the chromocentral model, is that when the *Y* chromosome is added to the female genome, it becomes part of the chromocenter and modifies the manner, or degree, of homolog pairing in this critical region. This, however, has been criticized by GRELL (1970)

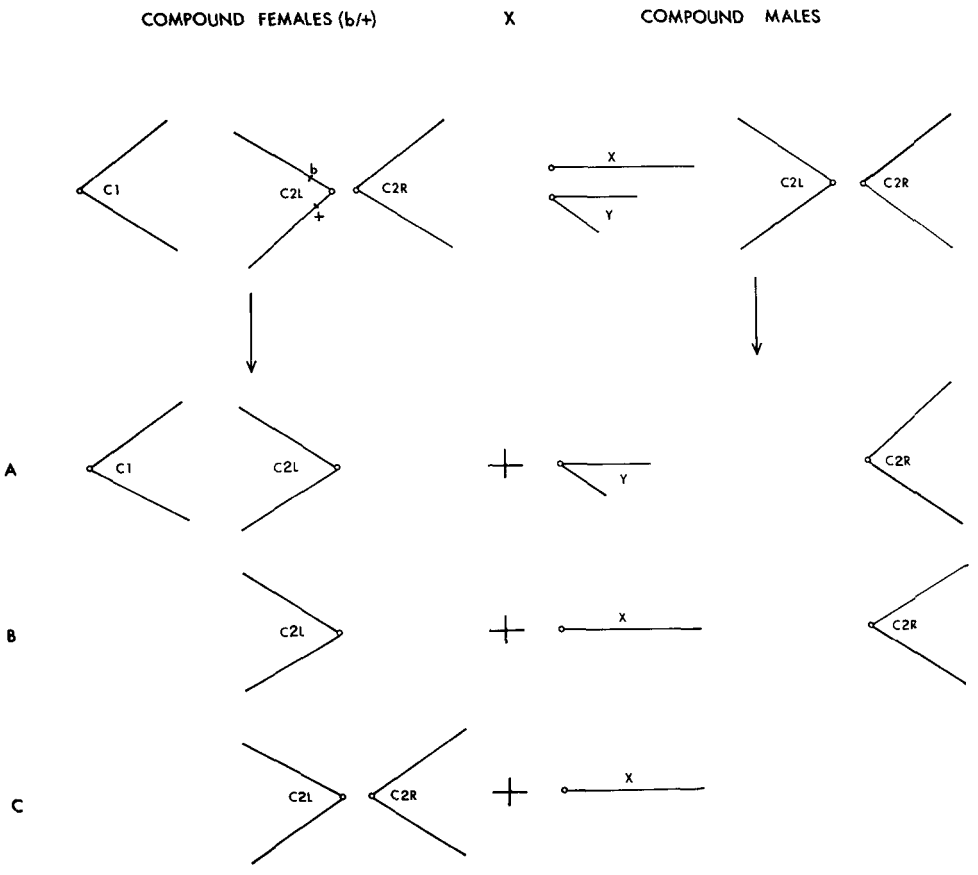

FIGURE 2.—The mating of females bearing three compound chromosomes (*C1*, *C2L*, and *C2R*) to males with two compounds in order to identify the three common segregational types from the females, all deriving *C2L* from the female parent. *C1* is marked with *yellow*, and, in the male, *C2L* carries dumpy, *C2R* carries cinnabar and brown. This makes the identification of all three segregational types unambiguous, at the same time that the frequency of homozygosity for black is recorded for each type.

on the grounds that the effect of an extra *Y* chromosome might, instead, be physiological in nature, with no relationship to any physical pairing phenomenon. This contention is difficult to counter, requiring some experimental set-up not involving the *Y* chromosome where frequencies of exchange might be modified depending upon some type of association of non-homologous chromosomes, this association leading to different segregation frequencies.

The configuration used for this purpose is shown in Figure 2. An attached-*X* female with two compound autosomal arms, *C(2L)* and *C(2R)*, was mated to appropriately-marked males, making it possible to identify the three common segregational types from that female. At the same time, a locus (black) near the centromere of *C(2L)* has been made heterozygous so that the frequency with which homozygosis occurs can be observed. There are two possible results from

this experiment. According to the distributive pairing hypothesis, these chromosomes will be involved in crossing over internally, and subsequently will pair "distributively" and segregate in different combinations into the various gametes. Since exchange occurs at the "exchange pairing phase", independent of distributive pairing, the frequency of homozygosis should be the same in all classes. On the other hand, the chromocentral hypothesis suggests the possibility of a slightly different result. At the time of chromocenter formation, these three compounds will be associated in a variety of ways from one cell to the next. In some of these configurations, exchange near the centromere will be inhibited, in others increased, depending upon the nature of the association. At the same time, that association may predetermine their mode of segregation. For this reason, different segregational products might show different degrees of homozygosis. This experiment was performed in triplicate, with three compounds of different origin in order to avoid the danger that some inherent peculiarity of one of the compounds might give rise to unusual products. Two aspects of this experiment should be emphasized. First is that the relative frequency of the different kinds of segregation is of no importance since we are concerned only with the relative frequency of homozygosis for the black allele within each segregational category. The second is that all compound arms are undoubtedly undergoing normal exchange; that is, this experiment is not making a distinction between compounds that are undergoing exchange and those that are not, but rather between those that have had a detectable exchange in the short genetic distance between black and the centromere of 2L and those that have not.

RESULTS

The results are given in Table 4. A chi-square is calculated for each experiment based on the hypothesis that all segregating classes should have the same frequency of homozygosis for black. The overall χ^2 suggests this to be very unlikely ($p < .01$).

TABLE 4

Frequencies of homozygosis for black in different segregational classes from females carrying three compound chromosomes shown in Figure 2

	Segregation types								
	A		B		C		Expt. totals		Contributions
Expt. no.	b^+	b	b^+	b	b^+	b	b^+	b	to χ^2
I Obs.	1172	46	1271	35	1651	41	4094	122	4.94
Exp.	1182.8	35.2	1268.2	37.8	1643.1	48.9			
II Obs.	1145	32	1501	25	1978	29	4624	86	7.14
Exp.	1155.5	21.5	1497.1	27.9	1970.4	36.6			
III Obs	1964	43	2386	69	3106	61	7456	173	5.11
Exp.	1961.5	45.5	2399.4	55.6	3095.2	71.8			
								Total χ^2	17.19
								$P < .01$	

A word is needed about the validity of the comparisons on which these conclusions are based. Whereas ordinarily an "experimental" set is compared with a control, run at the same time, and as similar to the experimental as can be managed, in the translocation experiment the results were matched against one ideal ratio (1:1:1:1) demanded by the distributive pairing hypothesis and a prediction (1:>1:>1:1) provided by the single pairing phase model. In the attached autosome experiment, the test is made for heterogeneity between three different segregational classes. Since all three classes come from the same females, genetic heterogeneity should be minimal and non-genetic heterogeneity should be limited to that found between eggs produced by the same female during the same period of time. These considerations suggest that both experiments, with their "internal" controls, are intrinsically more convincing than the usual experiment-control comparison.

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Corresponding editor: B. H. JUDD