DISTRIBUTIVE PAIRING: MECHANISM FOR SEGREGATION OF COMPOUND AUTOSOMAL CHROMOSOMES IN OOCYTES OF *DROSOPHILA MELANOGASTER*[1]

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THE segregational behavior of Drosophila chromosomes in the first meiotic division of the oocyte is quite different from that expected from the classical picture of the independent assortment of nonhomologous chromosomes and the regular segregation of homologous chromosomes. Under appropriate circumstances, nonhomologous chromosomes may segregate very regularly to opposite poles of the spindle of the first meiotic division, and conversely, homologs may frequently pass to the same pole (R. F. GRELL 1957, 1959, 1962a, 1962b, 1964a, 1964b). Simple rules which reliably predict the behavior of chromosomes in oocytes have been formulated and incorporated into the distributive pairing model of meiosis (R. F. GRELL 1962a, 1962b, 1964a, 1964b; E. H. GRELL 1963).

According to the distributive pairing hypothesis, after the crossing over process noncrossovers and compound chromosomes make up the "distributive pool." Chromosomes in this pool are distributed to the two poles without being influenced by homology but according to certain rules. The relative sizes of the members of the pool determine their segregation (see reviews by R. F. GRELL 1965, 1967). Nonhomologous distributive pairing occurs only in oocytes and does not occur during meiosis in males (R. F. GRELL 1957).

Compound autosomes are a class of useful and interesting chromosomes in Drosophila. They consist of two identical autosomal arms attached to one centromere, and are sometimes called isochromosomes in the cytological literature. They were first synthesized in the laboratory of E. B. LEWIS. Compound *2L*, compound *2R* and compound *3L* were synthesized by INGE RASMUSSEN; compound *3R* by E. ORIAS and P. DEAL (RASMUSSEN 1960); and compound *4* by LEWIS and A. ROBERTS. Compound autosomes have been used in a number of experiments. LEWIS (1967) used compound *3R* chromosomes to recover both reciprocal products of crossing over between pseudoalleles of the *bx* complex. RASMUSSEN (unpublished) recovered the h^2 *h* double mutant with a compound *3L*. HEXTER, LOZNER and BUNN (1967) obtained reciprocal crossovers between members of the *ss* complex. BATEMAN (1968) used compound autosomes to study radiation-induced nondisjunction and compound formation. MCCLOSKEY (1966) used them to demonstrate that sperm lacking a whole chromosome arm are functional. LINDSLEY and GRELL (1969) extended the demonstration to show that

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spermatids develop into functional sperm even when lacking both major autosomes. BALDWIN and CHOVNICK (1967) studied crossing over in a compound autosome and found it to be essentially normal. Several of the above authors have commented on some aspects of the segregational behavior of the compounds, but special attention to segregation has been given only by BALDWIN and CHOVNICK (1967) and by HOLM, DELAND and CHOVNICK (1967). In no previous report has it been acknowledged that the behavior of compound autosomes is expected on the basis of the distributive pairing model of meiosis. The purpose of this work, therefore, is to more fully describe the meiotic behavior of compound autosomes and to discuss their behavior in the framework of the distributive pairing hypothesis.

MATERIALS AND METHODS

Most of the mutants and chromosome rearrangements used in this investigation are described in LINDSLEY and GRELL (1968). Stocks of compound autosomes were originally obtained from E. B. LEWIS. Compounds with different markers or inversions were induced by irradiating females carrying the appropriate noncompound chromosomes and mating to males from a compound chromosome stock. The progeny are few since viable products are limited to compounds and nondisjunctional products (BATEMAN 1968). Flies with the desired markers were selected from among the progeny.

One chromosome used in this study has not been previously described. It is a free *2R* chromosome with all of the euchromatic part of *2R* and none of *2L*. It is given the symbol *F(2R)1*. Under LINDSLEY and GRELL's outlines for naming rearrangements, this chromosome would be called a *Df(2L)* since it is deficient for *2L* chromatin; but referring to it as a deficiency seems confusing, so the new symbol is introduced. *F(2R)1* is derived from a crossover between *In(2LR)lt*^{ms} and a normal-sequence chromosome. Its synthesis is diagrammed in Figure 1. *F(2R)1* is homozygous viable when present with a compound *2L* or two free *2L*'s. The two *F(2R)*'s disjoin regularly in both males and females. Crossing over appears to be in the normal range (*cn–c* 15.5 units; *c–bw* 25.4 units).

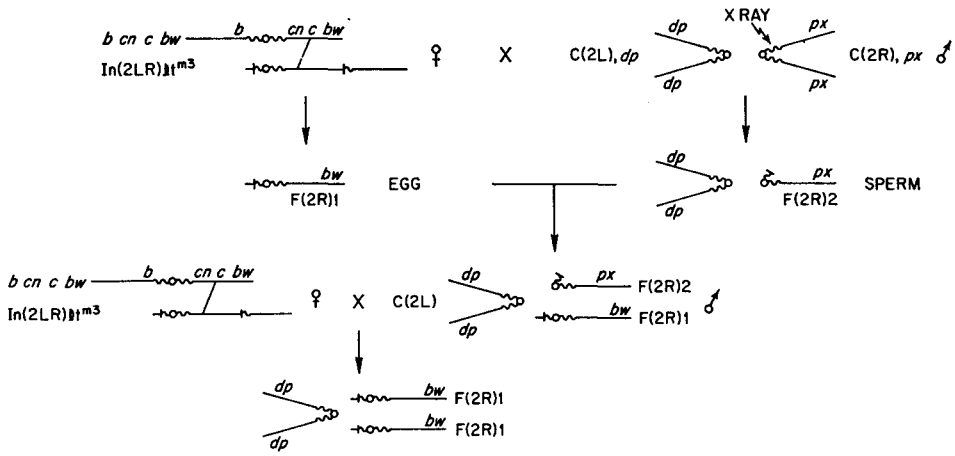

FIGURE 1.—Synthesis of *F(2R)1*. X-irradiated *C(2L), dp*; *C(2R), px* males were mated to *b cn c bw/In(2LR)lt*^{ms} females. Few progeny are produced since most zygotes are not viable. Male progeny that were *dp* — were selected. Each male was mated to *b cn c bw/In(2LR)lt*^{ms} females. Offspring of these crosses included *dp bw* males and females. They were selected to establish a stock of flies carrying *C(2L), dp* and homozygous *F(2R)1, bw*.

Drosophila were cultured on a cornmeal, sugar, dried brewer's yeast, and agar medium similar to the one described by LEWIS (1960). Temperature was maintained at 25°C; data were collected from single female cultures; and parents were removed after six days.

RESULTS

From the mating of *C(2L), cl; C(2R), cn* females with *C(2L), +; C(2R), sp^2 bs^2* males, two types of offspring are predominantly recovered. The majority type contains one maternal and one paternal compound chromosome. The minority type has two compounds from one parent and none from the other. The minority type (called exceptions here) occurred with a frequency of 0.010 (Table 1).

The presence of heterozygous inversion in one arm of the compound *2L* did not greatly affect the frequency of exceptions. Females with a heterozygous *In(2L)Cy* in one arm of the compound *2L* produced 0.012 exceptions.

A less regular segregation of the compounds is observed if the females are also heterozygous for an inversion in the other large autosome. In Table 2 are data from females heterozygous for *$In(3LR)Ubx^{130}$* or *In(3L)P + In(3R)C*. The frequency of the exceptions is 0.02 in the presence of heterozygous *In(3L)P + In(3R)C* and from 0.028 to 0.046 in the presence of heterozygous *$In(3LR)Ubx^{130}$*. Females heterozygous for *In(3L)P + In(3R)C/$In(3LR)Ubx^{130}$* produced 0.04 exceptions. Females containing a compound *2L* heterozygous for *In(2L)Cy* as well as a heterozygous inversion in chromosome *3* produced a frequency of 0.079 exceptions which is not significantly different from the 0.046 observed without the inversion in *2L*.

The introduction of a *Y* chromosome into *C(2L); C(2R)* females has a large effect on their segregation. The frequency of exceptions ranged from 0.238 to 0.300 (Table 3). It is also important that the segregation of the *Y* is correlated with the segregation of the compound autosomes. Offspring with both maternal compounds receive no *Y* chromosome and those with neither maternal compound receive the *Y* with only rare exception. In three of the four experiments there was a preference for the *2L⟷Y2R* segregation over the *2R⟷Y2L* segregation.

A more complicated situation is present in *C(1)RM/B^sY; C(2L); C(2R)* females. Again there is an appreciable frequency of eggs with both or no compound autosome. In this case the most common segregation that involves autosomal exceptions also involves the sex chromosomes. Offspring with both maternal autosomal compounds receive no *Y* or compound *X* and those with neither compound autosome tend to receive both the *Y* and compound *X* (Table 4). The proportion of eggs that receives other than two of these elements is fairly large (about 0.05).

A more simple situation is the one in which the female genotype is *+/+/Y; C(2L); F(2R)1/F(2R)1*. In these oocytes the *Y* and *C(2L)* are generally the only large chromosomes always in the distributive pool. The *Y* and *C(2L)* pass to opposite poles of the first meiotic division with a frequency of 0.971 (Table 5). Only 0.029 of the progeny carried both or neither chromosome.

The involvement between compound and noncompound nonexchange chromo-

TABLE 1

Offspring of parents with compound second chromosomes

Genotype of mother	Genotype of father	Chromosomes received from mother				nd* of
		C(2L)	*C(2R)*	*C(2L); C(2R)*	0	*C(2L)* and *C(2R)*
C(2L)+; C(2R)cn	*C(2L)cl; C(2R)sp² bs²*	1117	1362	5	21	0.010
C(2L)In(2L)Cy Bl; C(2R)cn	*C(2L)+; C(2R)px or*	318	447	2	7	0.012

* Nondisjunction frequency.

TABLE 2

Offspring of females with compound second chromosomes and heterozygous inversions in third chromosomes

Genotype of mother	Genotype of father	Chromosomes received from mother								nd* of
		C(2L); +	*C(2L); In*	*C(2R); +*	*C(2R); In*	*C(2L); C(2R); +*	*C(2L); C(2R); In*	*0; +*	*0; In*	*C(2L)* and *C(2R)*
C(2L)+; C(2R)cn; In(3LR)Ubx¹³⁰/+	*C(2L)dp; C(2R)px*	228	267	125	110	4	6	6	5	0.028
C(2L)dp; C(2R)cn; In(3LR)Ubx¹³⁰/+	*C(2L)+; C(2R)+*	177	176	197	209	10	2	9	16	0.046
C(2L)dp; C(2R)cn; In(3L)P+In(3R)C, Sb	*C(2L)+; C(2R)+*	117	108	116	109	1	0	4	4	0.020
C(2L)dp; C(2R)px; In(3LR)Ubx¹³⁰/In(3L)P + In(3R)C, Sb	*C(2L)nub² b pr; C(2R)cn*	192	182	245	223	6	9	10	11	0.041
C(2L) In(2L)Cy Bl; C(2R)px; In(3LR) Ubx¹³⁰/+	*C(2L)dp; C(2R)cn*	289	147	248	236	21	8	38	12	0.079

* Nondisjunction frequency.

TABLE 3

Offspring of females with a Y *chromosome and compound second chromosomes*

Genotype of mother	Genotype of father	Chromosomes received from mother									
		C(2L)	*C(2R)*	*Y; C(2L)*	*Y; C(2R)*	*C(2L); C(2R)*	*Y*	*Y; C(2L); C(2R)*	*0*	Total	nd* of *C(2L)* and *C(2R)*
B^sY; C(2L)In(2L)Cy Bl; C(2R)+	*C(2L)+; C(2R)sp^2 bs*	285	195	122	287	218	166	3	4	1280	0.31
B^sY; C(2L)In(2L)Cy Bl; C(2R)+	*C(2L)nub^2 b pr; C(2R)cn*	70	46	35	77	48	29	0	0	305	0.25
B^sY; C(2L)+; C(2R)+	*C(2L)nub^2 b pr; C(2R)cn*	64	37	47	68	40	47	1	1	305	0.29
B^sY; C(2L)dp; C(2R)+	*C(2L)+; C(2R)cn*	107	114	82	96	60	65	1	0	525	0.24

* Nondisjunction frequency.

TABLE 4

Offspring from C(1)RM, y/B^{s}Y; C(2L), +; C(2R), + *females* × C(2L), nub^{2} b pr; C(2R), cn *males*

Chromosomes received from mother	Experiment A	Experiment B
C(1); C(2L)	150	160
C(1); C(2R)	113	141
C(1)/Y	13	33
C(2L); C(2R)	43	23
Y; C(2L)	153	141
Y; C(2R)	96	143
C(1)	3	2
Y	5	10
C(2L)	11	4
C(2R)	5	2
C(1)/Y; C(2L)	0	4
C(1)/Y; C(2R)	0	11
Y; C(2L); C(2R)	6	2
C(1); C(2L); C(2R)	24	12
nd* of *C(1)* and *Y*	0.12	0.11
nd* of *C(2L)* and *C(2R)*	0.15	0.12

* Nondisjunction frequency.

TABLE 5

Offspring obtained from B^{s}Y; C(2L), dp; F(2R)1/F(2R)1; *females* × C(2L), +; F(2R)1/F(2R)1 *males*

Experiment	Chromosomes received from mother *Y; C(2L)*	*Y; 0*	*0; C(2L)*	*0; 0*	nd* of *Y* and *C(2L)*
A	5	112	150	10	0.054
B	10	482	631	16	0.023

* Nondisjunction frequency.

TABLE 6

Progeny of In(1)B^{M1}, y cv v/ y^{2} sc f; C(2L), dp; F(2R)1/F(2R)1 *females and* C(2L), +; F(2R)1/F(2R)1 *males*

Chromosomes received from mother *X; C(2L)*	*X; 0*	*XX; C(2L)*	*XX; 0*	*0; C(2L)*	*0; 0*	nd* of *X*'s
756	751	0	41	50	0	0.11

* Nondisjunction frequency calculated as two times exceptionals divided by (the sum of the regulars plus two times the exceptionals) in order to correct for lethality of *XXX* and *Y0* animals.

somes can be seen in the following experiment. Females of the genotype, y^2 *sc f/In(1)*B^{M1}, *y cv v; C(2L), dp; F(2R)1/F(2R)1* were constructed. The nondisjunction of the *X* chromosomes was always correlated with the segregation of *C(2L)* (Table 6). Eggs containing two *X* chromosomes never had the *C(2L)* and those containing no *X* chromosome always had the *C(2L)*. Male progeny were scored for crossing over between the *X* chromosomes. A value of 34.5% crossing over was calculated for the region outside of the inversion (*y* to *f*). The females with two maternal chromosomes were collected as virgins and thirty were successfully tested for possession of crossover chromosomes. No crossover chromosomes were found. As expected from the distributive pairing model, nonexchange chromosomes are exclusively able to interact with the compound chromosome to produce nondisjunction.

DISCUSSION

Compound autosomes are very susceptible to highly nonrandom assortment with nonhomologous, noncrossover chromosomes, as shown in these experiments; this susceptibility has been previously investigated with compound *X* chromosomes; and it was concluded that a compound chromosome is always a member of the distributive pool (E. H. GRELL 1963). The distributive pool is made up of (noncompound) chromosomes that are noncrossovers and all compound chromosomes, and their segregation is determined by the nature of the chromosomes in the pool. Chromosomes of similar size affect each other's segregation more frequently than chromosomes of different size (R. F. GRELL 1964a). If only two chromosomes are in the distributive pool, they pass to opposite poles of the first meiotic division spindle with regularity even though they are different in size. But if three elements of different size are in the pool, they tend to segregate so that the middle-sized element goes to one pole and the larger and smaller elements go to the other pole.

In females with only *C(2L)* and *C(2R)*, the two compounds pair during the distributive pairing phase and they are the only large chromosomes regularly in the distributive pool. Fourth chromosomes are also members of the pool since they are noncrossovers, but their size is different enough so that they do not interact with the compounds. The compounds segregate to opposite poles because of distributive pairing and not because of any homology that they might share as a consequence of both having a second chromosome centromere.

In males which do not have nonhomologous distributive pairing, *C(2L)* and *C(2R)* would be expected to segregate randomly or nearly so. All of our experiments indicate that sperm having neither or both compounds are produced in appreciable frequencies, the principal indication being that in the presence of a *Y* chromosome, *C(2L)* and *C(2R)* appear to segregate nearly at random in the female. A large nonrandomness of *C(2L)—C(2R)* segregation in the male, however, would not permit this. A similar conclusion about the segregation of compounds in males was reached by BALDWIN and CHOVNICK (1967) and by BATEMAN (1968).

BALDWIN and CHOVNICK (1967) and HOLM, DELAND and CHOVNICK (1967)

reported increases of eggs containing neither and both *C(3L)* and *C(3R)* in the presence of a heterozygous inversion of the second chromosome. In data presented above there is a similar effect on *C(2L)—C(2R)* segregation by the presence of heterozygous inversions in the third chromosome. The effect of the nonhomologous inversion on compounds is to be expected on the basis of distributive pairing. The heterozygous third chromosome inversion causes many nonexchange tetrads of that chromosome. These third chromosomes then become members of the distributive pool where they may interact with the compound second chromosomes and cause a less regular segregation of the compounds. In this experiment only regular segregation of the third chromosomes gives a viable product. Therefore, only segregations in which one of the third chromosomes passed to one pole and three other chromosomes to the other pole could be recovered. A segregation in which the two compound second chromosomes go to one pole and the two third chromosomes go to the other is probably more frequent but was not recovered because of its lethality in this experiment.

With a heterozygous inversion in a nonhomologous chromosome, two more chromosomes were added to the distributive pool. By constructing females with a *Y* chromosome, which is always a noncrossover and always a member of the distributive pool, one may also add only one chromosome. This addition had a very large effect on the segregation of the compounds. *C(2L)* and *C(2R)* go to the same pole of the meiotic spindle in 0.238 to 0.300 of the oocytes. The *Y* is nearly equivalent to a compound. If it were entirely equivalent, that segregation would make up 0.33 of the total. In three of the four experiments there is a marked excess of the *C(2L)* ⟷ *Y; C(2R)* segregation over the *C(2R)* ⟷ *Y; C(2L)* segregation. This kind of result has also been obtained in situations where there are three chromosomes of unequal size in the distributive pool. The preferred segregation is the one in which the middle-sized chromosome goes to one pole and the smallest and largest go to the other pole (R. F. GRELL 1964a).

HOLM *et al.* (1967) mention that the presence of a compound *X*, *FMA3* [called *C(1)M3* in LINDSLEY and GRELL (1968)] causes nondisjunction of *C(3L)* and *C(3R)*, that eggs which have both compound autosomes tend not to carry the compound *X*, and that eggs with the compound *X* tend not to carry the compound autosomes. In the experiment reported here, the compound *X* is a simple reversed metacentric, the traditional attached *X*. It contains no inversions as does *FMA3* and the arms are free to cross over as with normal *X* chromosomes. The *Y* chromosome was marked with B^s (BROSSEAU, NICOLETTI, GRELL and LINDSLEY 1961) so that it may be followed in the segregation. Since the *Y* is a member of the distributive pool, it participates in the pairing that determined the segregation. There is an increase in *C(2L)—C(2R)* nondisjunction in the presence of *C(1)RM/Y* and furthermore there is a tendency for nondisjunction of the sex chromosomes to accompany nondisjunction of the compound autosomes such that eggs carry both compound autosomes and neither sex chromosome, or the reverse. The tendency is for two chromosomes to go to each pole of the first meiotic division spindle although there are between 0.05 and 0.06 three-to-one segregations.

A fairly regular segregation of a compound autosome and a *Y* chromosome

should be possible if they are the only two large chromosomes in the distributive pool. They should nearly always be directed to opposite poles of the first meiotic division spindle. Up to this point there were always two compound autosomes present in the experimental flies. One compound may be eliminated by substituting two free chromosome arms in its place. The free arms are usually crossovers and hence usually not members of the distributive pool. In these experiments flies of the genotype, *B^sY; C(2L), dp; F(2R)1/F(2R)1*, were constructed. The *F(2R)*'s in the distributive pool, however, are very likely the cause of the nondisjunction of *Y* and *C(2L)* in 2.3 to 5.4% of the oocytes. *F(2R)1* has about 20% noncrossover tetrads, and these would tend to prevent an entirely regular segregation of *C(2L)* and *Y*.

The experiment in Table 6 demonstrated several points. A heterozygous *X* inversion and a compound *2L* were used in the same way that a heterozygous *X* inversion and a *Y* was used by R. F. GRELL (1962b). Without a *Y* or *C(2L)* heterozygous *In(1)B^{M1}* yields 0.0035 nondisjunction; with a *Y* there is 0.185 nondisjunction. In the experiment with *C(2L)* there was 0.108 nondisjunction of the *X*'s. Crossing over was the same with the *Y* and *C(2L)*. The difference in nondisjunction frequencies must not be a reflection of a difference in the frequency of *X* chromosomes in the distributive pool, but rather it must be that *C(2L)* is less efficient than the *Y* in causing the *X*'s in the pool to nondisjoin. The presence of some *F(2R)*'s in the distributive pool also tends to reduce the *X* nondisjunction frequency. The nondisjunction of the *X*'s caused by *C(2L)* is a case of distributive nondisjunction (R. F. GRELL 1970). Secondary nondisjunction is distributive nondisjunction caused by a *Y* chromosome. Nondisjunction of *X*'s caused by a heterozygous autosomal inversion is another example of distributive nondisjunction and has been considered in the context of the distributive pairing hypothesis (ROBERTS 1962).

SUMMARY

Compound autosomes of Drosophila assort nonrandomly with certain nonhomologous chromosomes in oocyte meiosis. In terms of R. F. GRELL's distributive pairing hypothesis, the compound autosome is always a member of the distributive pool and therefore always available for nonhomologous distributive pairing. If two compound autosomes are the only members of the distributive pool, they regularly segregate to opposite poles of the meiotic spindle. If a *Y* chromosome constitutes a third member of the distributive pool, it participates in the segregation process and causes about 30% nondisjunction of the two compounds. If only one compound autosome and a *Y* are present in the pool they segregate to opposite poles in more than 95% of the oocytes.—It was also demonstrated that a noncrossover *X* chromosome can pair with a compound autosome but a crossover *X* does not. This behavior is again an expectation of the distributive pairing hypothesis.

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