all of the multiple enzyme types resulting from the treatment are more negatively charged than the original enzyme. With the exception of an enzyme that migrates slower than SS, the converted enzymes have migration rates identical to the auto- and allodimer isozymes occurring in the homozygous and heterozygous combinations of the E_1^F, E_1^N, and E_1^S alleles. It is postulated that these three alleles specify polypeptides with identical charges but differing in the number of conjugated positively charged side groups. Loss of varying numbers of these side groups after treatment with $NaBH_4$ is proposed to explain the isozyme interconversions.

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*DISTRIBUTIVE PAIRING: THE SIZE-DEPENDENT MECHANISM FOR REGULAR SEGREGATION OF THE FOURTH CHROMOSOMES IN DROSOPHILA MELANOGASTER**

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The fourth chromosome of *Drosophila melanogaster* occupies a unique position in the genome for, in addition to its extremely small size, it fails under normal conditions to undergo exchange. It has been shown that when the remainder of the Drosophila chromosomes are nonrecombinant during oögenesis, they become available for nonhomologous associations at the time of distributive pairing.[1] Despite the fact that the fours are noncrossovers, the evidence indicates that they do not participate in associations with heterologues. Failure to detect such associations, unless one fourth chromosome is effectively absent through involvement in a translocation or unless an extra free four is present, originally led to the supposition that a special device, peculiar to the fours, might be responsible for their regular behavior. The recent finding[2] that pairing preference during the distributive process is size-dependent suggested, as an alternative explanation, that it is the minute size of the fours which normally safeguards them from heterologous involvement. If the failure of a nonrecombinant heterologue to associate with the fours is due to the size disparity between the fours and the other chromosomes of the

genome, a heterologue more or less equivalent in length might be expected to achieve such an association and lead to fourth chromosome nondisjunction. Experiments have been carried out with the purpose of testing this premise. The results indicate that the fourth chromosomes are members of the distributive pool and that they will, like other chromosomes in the pool, associate nonhomologously when a heterologue of the proper size is present. It follows that no mechanism peculiar to the fours need be invoked to account for their regular segregation under most conditions.

Materials and Methods.—A number of the free X duplications that were produced and extensively characterized, both cytologically and genetically, by Cooper and Krivshenko (unpublished) and which have been briefly described previously,[2] served as the nonrecombinant heterologues for the fourth chromosomes. Each of the duplications carry the distal euchromatic tip of the X, including the locus of yellow$^+$ (y^+), and variable amounts of proximal X heterochromatin. If the length of chromosome four at mitotic metaphase is taken as unity, the lengths of the X duplications relative to chromosome four vary, according to the determinations of Cooper and Krivshenko, from ≤ 0.3 to 3.3. A series of females was constructed of the genotype y^2/y^2/Dp(1;f), y^+; ci^D/ey^D. Genotypes differed from one another only by the particular X duplication present. It should be noted that the ey^D genotype is associated with a euchromatic duplication in chromosome four so that the ey^D fourth chromosome is somewhat larger than the nonduplicated ci^D fourth chromosome. These females were mated to males bearing both an attached XY chromosome marked with yellow (y) and Bar (B) and a free unmarked Y. The presence of y^+ on each duplication and of the dominant markers cubitus-interruptus-Dominant (ci^D) and eyeless-Dominant (ey^D) on the two free fours permitted the segregation of the three relevant chromosomes in the mother to be followed among the progeny. As a control, the frequency of fourth chromosome nondisjunction was measured among the progeny of females of otherwise identical genotype that lacked an X duplication.

In an independent experiment, progeny from females of the genotype y^2/y^2; T(3;4)86D/Tp(3)-Vno, *Vno*;ci^D/ey^D were studied. In this case one of the two products of the reciprocal translocation acted as a nonrecombinant heterologue in place of an X duplication. This chromosome, designated T_4, carries the centromere and proximal heterochromatin of chromosome four and the distal two thirds of the euchromatin of the right arm of three. At mitotic metaphase it appears 5–6 times the length of chromosome four. The presence of the dominantly marked transposition Tp(3)Vno, *Vno* in the distal portion of the right arm of the nontranslocated third chromosome reduces crossing over between T_4 and the nontranslocated 3 to less than 2%.

In each experiment, nondisjunction values were calculated for the three chromosome sets, (1) ci^D,ey^D; (2) Dp,ci^D or T_4,ci^D; and (3) Dp,ey^D or T_4,ey^D. Viability corrections were made only for the haplo-4 offspring, and these corrections were based on the frequency of the reciprocal triplo-4 class. Since the doubly mutant triplo-4 flies ($ci^D/ey^D/+$) are probably the least viable among the nonhaplo-4 progeny, the nondisjunction values for the fourth chromosomes are minimal ones.

In all experiments virgin females, within 10 hr after eclosion, were placed singly in vials with three males. After 24 hr the parents were transferred to bottles where they were kept for 6 days and then discarded. The temperature was maintained at 25° ± 1°C throughout.

Results and Analysis.—The frequencies of the four possible types of segregation of the two fourth chromosomes and the duplication (or T_4) are given in Table 1, columns 4, 5, 6, and 7. Nondisjunction values for the three chromosome sets are practically identical with the frequencies of the three principal types of segregation (Table 1, columns 4, 5, and 6) since the frequency of the fourth segregation type (column 7), which is simultaneously nondisjunctional for all three chromosomes, is negligible.

The nondisjunction frequencies for the three sets of chromosomes [ci^D,ey^D; Dp (or T_4),ci^D; Dp(or T_4),ey^D] are plotted as a function of the length of the heterologue (Dp or T_4) in Figure 1. The curve for fourth chromosome nondisjunction,

TABLE 1

SEGREGATION FREQUENCIES OF ci^D,ey^D AND DUPLICATION CHROMOSOMES IN PROGENY FROM CROSSES OF $y^2/y^2/Dp(1;f),y^+$; ci^D/ey^D ♀ ♀ × $\overline{XY}$, yB/Y ♂ ♂

			Progeny Recovered from Different Types of Segregation (%)			
Dp no.	Dp length	Totals*	Dp,ci^D ↔ ey^D	Dp,ey^D ↔ ci^D	ci^D,ey^D ↔ Dp(M)†	Dp, ci^D,ey^D ↔ O(M)†
1187	≤0.3	2,078	44.8	55.0	0.2	0.05
1162	0.5	1,693	43.4	52.8	3.8	0.07
816	0.7	3,663	34.1	55.9	10.0	0.05
1204	0.9	2,039	27.6	53.5	18.9	..
1193	1.0	2,806	35.6	41.6	22.8	..
1144	1.1	3,270	26.4	37.0	36.6	..
1339	1.1	2,126	44.6	37.9	17.5	0.05
1337	1.4	1,543	53.7	34.4	11.9	..
1186	1.6	3,011	47.5	38.4	14.2	..
1346	2.0	2,399	55.1	36.3	8.6	..
1328	2.1	844	64.2	32.0	3.8	..
1488	2.5	3,624	60.7	35.5	3.8	..
856	3.0	2,297	60.4	37.3	2.4	0.05
1498	3.3	752	59.6	37.2	3.2	..
T_4	5–6	1203	59.3	40.5	0.3	..

* Totals inflated by correction for inviability of haplo-4 progeny.

† (M) = Minute = haplo-4; a viability correction based on the reciprocal triplo-4 progeny has been made for haplo-4's.

A control experiment using y^2/y^2; ci^D/ey^D females produced two $ci^D/ey^D/+$ individuals among 1,860 flies which, after correction for the reciprocal, haplo-4 class, is 4/1,862 or 0.2% fourth chromosome nondisjunction.

originating at 0.2 per cent for the smallest duplication (1187 = ≤ 0.3) and identical to the control value, rises steeply as the length of the duplication approaches the length of the fourth chromosomes. At approximate equality between the duplication and the fours (Dp 1144 = 1.1), ci^D,ey^D nondisjunction reaches a maximum of 36.3 per cent, whereas nondisjunction between the duplication and ci^D is only 26.4 per cent. At this point segregation of the two heterologues, the X duplication and ci^D, is, therefore, more regular than that of the homologues ci^D and ey^D. Beyond this point, as the duplication becomes larger than either four, fourth chromosome nondisjunction abruptly decreases and tapers off with increasing duplication size, reaching a low of 0.3 per cent with T_4, the largest heterologue tested. The sharp decline beyond 1.1 suggests that at the time of distributive pairing, ey^D is much closer to ci^D in length than it is in salivary gland cell chromosomes, where ey^D is approximately twice as long as a fourth chromosome lacking the ey^D duplication. It should be noted that a second duplication (1339), also measured at 1.1, is, as judged by segregation frequencies, clearly not identical in length with 1144 and has been placed slightly to the right at 1.1+.

Nondisjunction values significantly in excess of 50 per cent mean that trivalents must be occurring. It has been shown that disjunction from trivalents composed of three unequal-sized heterologues follows a pattern in which the chromosome of intermediate length directs the small and large chromosomes to the same pole.[2] Here we see evidence for the same phenomenon. With duplications smaller than 1.0 and larger than 1.6, nondisjunction values greater than 50 per cent are observed. If the assumption, based on cytological grounds, is correct that the duplicated ey^D fourth chromosome is somewhat larger than the nonduplicated ci^D chromosome at the time of distributive pairing, for duplications smaller than the fours, ci^D will be the intermediate element and Dp,ey^D nondisjunction is expected to exceed 50 per cent; for duplications larger than the fours, ey^D will be intermediate in size, and Dp,ci^D nondisjunction is expected to exceed 50 per cent. In Figure 1, we see that

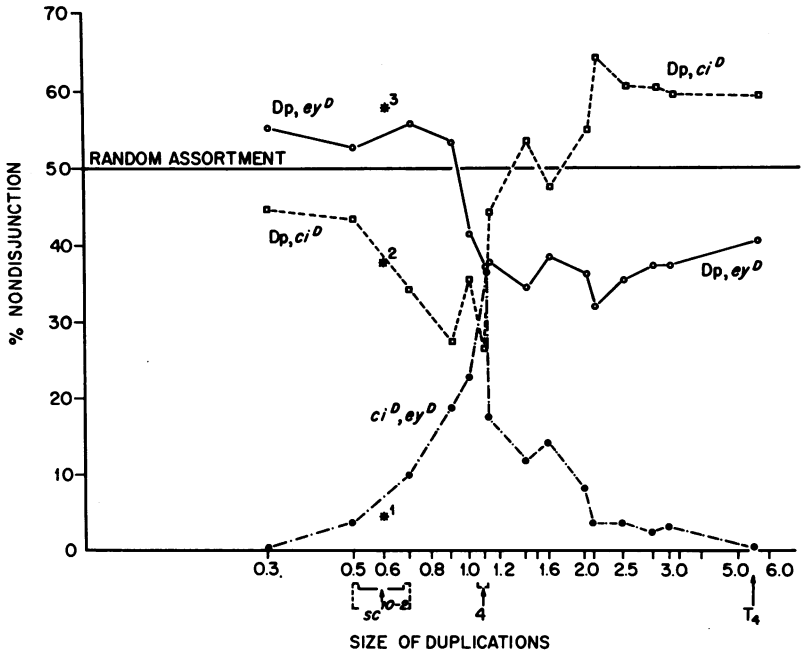

FIG. 1.—Nondisjunction frequencies for the three chromosome sets: (*1*) ci^D,ey^D; (*2*) Dp, ci^D or T_4, ci^D; (*3*) Dp,ey^D or T_4,ey^D plotted as a function of duplication (and T_4) length. The asterisk indicates the nondisjunction frequencies obtained by Sturtevant with the sc^{10-2} duplication where $*^1$ = ci^D,ey^D nondisjunction; $*^2$ = Dp,ci^D nondisjunction; $*^3$ = Dp,ey^D nondisjunction.

both expectations are realized. This result constitutes an independent genetic confirmation, that the ey^D chromosome is larger than the ci^D chromosome at the time of distributive pairing. It also means that some trivalent association between the fours and the smallest duplication as well as between the fours and T_4 must be inferred despite the failure of either heterologue to increase fourth chromosome nondisjunction.

In general, for duplications 1.0–1.6 in length, nondisjunction does not exceed 50 per cent so that trivalent formation need not be postulated although trivalents may well occur. The exception to this, Dp 1337 (= 1.4), for which a Dp,ci^D nondisjunction value of 53.7 per cent does imply trivalents, is highly inviable in combination with ey^D, so that this may represent a magnification of the true segregation frequency. The sharp fluctuations in the slopes of the curves between 0.9 and 1.1+ indicate that segregation behavior within this range, where the three chromosomes are most nearly equivalent, is very responsive to slight changes in size. Further study is needed to elucidate the segregation patterns here.

Discussion.—The present studies show that the fourth chromosomes, as nonrecombinants in the diploid female, are always members of the distributive pool and will associate nonhomologously if a heterologue of the proper size is simultaneously available. Under normal conditions the X chromosomes are also constituents of the pool about 5 per cent of the time, but with rare exception, distributive pairing, as judged by the frequencies of X and fourth chromosome nondisjunction, occurs exclusively between homologues.[3] This is equally true if the X chromosomes are

members of the pool over 90 per cent of the time as happens when the X's are heterozygous for Ins(1)dl-49, B^{M1}.[4] If a small X duplication is present in the pool, as is the case in the present experiments, associations between the duplication and the fours do occur. The frequency of these associations appears to be positively correlated with the similarity in size between the heterologues as indicated by the rapid increase in fourth chromosome nondisjunction when duplication size approaches chromosome four size from either direction. It follows from this that distributive pairing is the normal mechanism for regular segregation of the fourth chromosomes.

The present results raise the interesting question of whether the phenomenon of preferential segregation, discovered and investigated by Sturtevant,[5, 6] has as its basis a size difference between chromosomes. Sturtevant found that in triplo-4 females, the three types of segregation giving one fourth chromosome to one pole and two to the other do not occur with equal frequency. An order of preference was established such that any particular fourth chromosome would pass to the diplo-4 pole more frequently than any chromosome lying below it in the series. This seriation was demonstrated most satisfactorily when one of the three chromosomes was the sc^{10-2} duplication which carries a segment of X on the four base. An examination of the order established by the sc^{10-2} tests discloses that the duplicated ey^D chromosome lies fourth from one end of the series of 26 chromosomes, the Minute-4 chromosome, which possesses a large deletion, lies next to last at the other end, and the ci^D chromosome lies close to the midpoint. The positions of the duplicated and deleted chromosomes in conjunction with the genetic evidence, afforded by the present experiments that the ey^D chromosome is larger than the ci^D chromosome, suggest that the seriation could be a reflection of size difference among the fours such that the M-4 chromosome represents one of the smallest and the ey^D, one of the largest of the fourth chromosomes.

In a test, identical to that employed here, Sturtevant measured the segregation of the sc^{10-2} duplication with ci^D and ey^D. The following frequencies for the three principal types of segregation were obtained, $ci^D,ey^D \leftrightarrow sc^{10-2} = 4.2$ per cent; $ey^D,sc^{10-2} \leftrightarrow ci^D = 57.9$ per cent; and $ci^D,sc^{10-2} \leftrightarrow ey^D = 37.8$ per cent. The value of 4.2 on the ci^D,ey^D curve is consistent with a duplication size between 0.5 and 0.7, or with one between 2.0 and 2.1, but the two additional values definitively place the duplication at the former position (see Fig. 1). From its cytological description,[7] the duplication is expected to be smaller rather than larger than chromosome four and fits a position predicted by its genetic activity below 1.0. Thus, upon the basis of size, this duplication would constitute the lowest member of a series including the different fourth chromosomes in contradistinction to the highest member in the series constructed by Sturtevant on the basis of its recovery at the diplo-4 pole over 95 per cent of the time. It should be noted that an identical seriation for the fours is obtained whether the duplication lies at the bottom of the series and an intermediate element tends to be recovered at the haplo-4 pole or whether the duplication lies at the top and an element low in the series is recovered at the haplo-4 pole.

The behavior of three fourth chromosomes, like the behavior of duplications 0.9 to 1.1+ in length (Fig. 1) (i.e., precisely that size range within which the fourth chromosomes are expected to lie) is difficult to interpret and, as noted above,

requires further study. Nevertheless, the evidence suggests that chromosome behavior within this range, whatever the pattern of pairing and segregation, is size-dependent since it is those duplications which are closest to the four in size that have the greatest effect on fourth chromosome nondisjunction.

The seriation established by the sc^{10-2} duplication appears to be, for the few points in common, in good agreement with the seriation based on size observed in the present work. These results suggest that preferential segregation arises from slight size differences among the fourth chromosomes, and it is these differences which determine their association and disjunction patterns at distributive pairing and lead to their nonrandom segregation. Implicit in this hypothesis is the assumption that each four, for which an individual segregation pattern exists, is slightly different in size from all others in the series. It is conceivable that with the elimination of crossing over and the failure to obtain a shifting back and forth of chromatin material that would tend to equalize chromosome length, changes within the chromosomes, particularly in heterochromatic regions, would accumulate. Heterochromatic regions may contribute more length and euchromatic regions less to the chromosome at the time of distributive pairing than their salivary lengths would indicate. The ey^D chromosome, which carries about twice as much euchromatin as the ci^D chromosome in salivary cells, behaves as if it were very close to the ci^D chromosome at distributive pairing and cytologically at metaphase in larval brains ey^D is, according to Bridges,[8] only slightly larger than a nonduplicated fourth chromosome. If heterochromatic regions do contribute disproportionately to size at distributive pairing, then changes within these regions would be highly effective in altering chromosome size at this time.

The principle of chromosome behavior based on size appears to be equally valid when three heterologues are involved as previously demonstrated,[2] when two homologues and a heterologue are involved as is the situation in the present case, and, if the extrapolation to preferential segregation should prove correct, when three homologues are involved. It follows that homology is of negligible importance in distributive pairing.

Summary.—To test the possibility that the failure of a noncrossover chromosome to disturb the regular segregation of the two nonrecombinant fourth chromosomes at distributive pairing is due to the difference in length between the minute fourth chromosomes and the remaining chromosomes of the genome, a series of free X duplications, ranging in size from ≤ 0.3 to 3.3 times the length of chromosome four, have been introduced, one at a time, into females of the genotype y^2/y^2; ci^D/ey^D and fourth chromosome nondisjunction frequencies have been measured among the progeny. The following conclusions have been drawn:

(1) The fourth chromosomes are subject to the same rules of behavior during distributive pairing as the other chromosomes of *Drosophila melanogaster*. In the diploid female the fours, as noncrossovers, are virtually always members of the distributive pool and will associate nonhomologously if heterologues of similar size are also present in the pool.

(2) The frequency of association between the fours and a heterologue, as measured by fourth chromosome nondisjunction frequency, is closely correlated with the similarity in length between the fours and the heterologue.

(3) When the only heterologue available is a chromosome of normal size, the small size of the fours serves to ensure regular fourth chromosome segregation.

(4) Distributive pairing is the normal mechanism for regular segregation of the fourth chromosomes.

It is suggested that the basis for preferential segregation may be small differences in size among normal fourth chromosomes.

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[4] Cooper, K. W., *Genetics*, **30**, 472 (1945).

[5] Sturtevant, A. H., these PROCEEDINGS, **20**, 515 (1934).

[6] Sturtevant, A. H., *Genetics*, **21**, 444 (1936).

[7] The sc^{10-2} duplication arose as a reciprocal translocation between the X and fourth chromosomes with a break in four between the known loci and the spindle attachment and a break in X at 1E so that the base of four is either tipped with this segment of X or, if four breaks occurred as suggested by Schultz,[6] part of this X segment is intercalated between the two ends of four in place of the loci for all of the known fourth chromosome genes. The duplication is now lost.

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A POSSIBLE FUNCTIONING IN VIVO OF PLASTOCYANIN IN PHOTOSYNTHESIS AS REVEALED BY A LIGHT-INDUCED ABSORBANCE CHANGE

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Plastocyanin, a protein containing two atoms of copper per molecule, was discovered by Katoh[1, 2] and found to be localized in chloroplasts of green cells. Non-photosynthetic tissue of plants and photosynthetic bacteria do not contain this protein.[2] The copper of plastocyanin occurs in the ratio of 1 atom to about 300 molecules of chlorophyll and accounts for about 50 per cent of the total in the chloroplast. Katoh and Takamiya have recently shown[3] that extracted and purified plastocyanin could be reduced in the light when added to spinach chloroplast preparations. The rate of plastocyanin reduction was comparable to rates of reduction commonly seen with other added Hill reagents such as ferricyanide or indophenol dyes. Moreover, reduced plastocyanin was also photooxidized by digitonin-treated chloroplasts. Katoh and Takamiya[3] did not consider it likely "that the actual part, if any, played by plastocyanin in photosynthesis is that of a natural electron acceptor for the Hill reaction." Kok[4, 5] suggested that a copper-containing chloroplast enzyme such as plastocyanin could mediate the transfer of electrons between the two light reactions of photosynthesis.