

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

F. B. MUMFORD, *Director*

A Study of Factors Influencing Chromosomal Segregation in Translocations of *Drosophila Melanogaster*

H. BENTLEY GLASS

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SUMMARY

1. Segregation has been studied in the mutual translocations V_3 , V_4 , V_5 , and in the compound V_5/Gr , in *Drosophila*.
2. In all cases, including those studied by Dobzhansky, and one case in maize, an equality of axes of the cross-figure produces equality of the two aneuploid types, and inequality of the axes produces a predominance of the aneuploid type corresponding to segregation along the greater axis.
3. Reduction of the frequency of 2-chromosome aneuploid gametes in V_5/Gr indicates that acentricity of the intersection, by reducing competitive pairing, leads to a less random segregation.
4. Spindle-fiber homologies are not a disproportionate factor in determining segregation. Nor does the position of the spindle-fibers with respect to the position of the translocation point have any apparent effect.
5. These facts lead to the conclusion that segregation in general is determined by the forces exerted between homologous loci.
6. In the V_5/Gr configuration elimination of competitive pairing between two chromosomes fails to produce regular disjunction of these chromosomes from their homologues. This is taken to support the hypothesis that segregation tends to take place through the determination of an axis of disjunction dividing the chromosomes into numerically equal groups, followed by random assortment of the chromosomal pairs lying along this axis.
7. This hypothesis also accounts for the commonly observed 1 : 1 orthoploid : aneuploid ratio in translocations of *Drosophila*, *Zea*, *Pisum*, and other forms; and explains why factors which greatly alter the relative frequencies of the aneuploid types fail to affect the total proportion of orthoploid gametes.
8. Inversions affect segregation only when they lie preponderantly along one axis. In such cases their effect is to inhibit disjunction along the axis upon which they lie. This may be explained as due to the determination of the axis of disjunction according to the relative paired lengths of the several axes.
9. The relation of crossing-over to segregation is only secondary. Synapsis cannot be the common immediate cause of both. This is discussed in connection with the ideas of Darlington upon the origin and behavior of chiasmata.
10. Various possible explanations for the highly orthoploid segregation found in *Oenothera* and similar forms are discussed.

A Study of Factors Influencing Chromosomal Segregation in Translocations of *Drosophila Melanogaster*

H. BENTLEY GLASS*

INTRODUCTION

The underlying causes of the orderly segregation of chromosomes are still largely unknown. In this connection, the segregation of the modified chromosomes resulting from mutual translocation offers the readiest means of analysis of the principles governing the process of chromosome disjunction.

Such cases are widespread in nature and occur frequently after x-ray treatment, both in plants and animals. In *Datura*, chromosome rings occurring at metaphase in meiosis were interpreted by Belling (1927) as cases in which "segmental interchange" had occurred between non-homologous chromosomes. Håkansson (1928), Blakeslee (1929), and Darlington (1929a) suggested that similar rings in *Oenothera* are also to be interpreted as due to heterozygous translocations. The work of Cleland and Blakeslee (1930; 1931), Cleland (1932), and of Sturtevant (1931), Emerson (1931), and Emerson and Sturtevant (1931) has clearly substantiated this, by numerous successfully fulfilled predictions. [For a review of this extensive subject see Muller (1930) and Cleland (1933).] Other metaphase ring formations have been similarly interpreted in *Zea* (McClintock 1930; Burnham 1930, 1932; Brink and Cooper 1931, 1932); in *Tradescantia* (Darlington 1929b; Sax and Anderson 1933); in *Pisum* (Håkansson 1931, 1934; Pellew and Sansome 1931; Sansome 1932, 1933); in *Rhoeo* (Sax 1931; Sax and Anderson 1933); and in *Campanula* (Gairdner and Darlington 1931).

Meanwhile, translocations had been studied in *Drosophila* for some time, and naturally comparisons were made with the *Oenothera* situation. Muller (1930) studied a single translocation in *Drosophila*. Having already pointed out the essential role of balanced lethal factors, and of the linkage between these and heterozygous gene complexes, he now extended this concept to include interchromosomal linkage, brought about by means of segmental interchanges. Occasional exceptions to this linkage would produce homozygotes, the so-called "mutants". Sturtevant and Dobzhansky (1930), studied reciprocal translocations in *Drosophila*, comparing them with *Oenothera*. They observed

*National Research Fellow in the Biological Sciences, Dept. of Field Crops, Missouri Agricultural Experiment Station, Columbia, Missouri

an essential similarity, especially for *Drosophila* types where both breaks occurred adjacent to the spindle-fiber attachments.

Two fundamental differences were apparent, however. In the first place, the aneuploid gametes (i.e., those which do not contain a normal haploid complement of all genes, but carry duplications and/or deficiencies of some) are fertile in *Drosophila*, and the zygotes formed by the union of complementary aneuploid gametes are viable; while such gametes are lethal in plants, where there is a more complex haploid stage of several cell-generations.

Secondly, the percentage of aneuploidy in *Drosophila* is about 50% (and this holds true also for the translocations reported in *Zea*, *Pisum*, and *Tradescantia edwardsiana*); but in *Datura*, *Oenothera*, *Campanula*, *Tradescantia reflexa*, and *Rhoeo* it varies widely from this value. Blakeslee (1927, 1929) found that in *Datura* (in some instances) plants with chromosome rings do not produce sterile pollen. The directed segregation (i.e., the preferential formation of orthoploid gametes, containing a complete haploid complement of genes) which is presumably responsible for this was demonstrated cytologically in *Oenothera*. Irregularities in the zig-zag orientation of the chromosomes in the rings, when aligned on the metaphase spindle, vary in percentage from 0.0 (acuens-stringens) to a maximum of 53.85% (truncans-Hookeri). (Cleland and Oehlkers 1930).* It further appears, "dass mit Zunahme der Kettengrösse auch die morphologisch sichtbaren Unregelmässigkeiten in der Anordnung zunehmen". Thus, the 53.85% maximum of aneuploidy occurs in a ring of 14 chromosomes; while the highest value for a single ring of 4 chromosomes is 6.4% (flavens-stringens); and the minimum seems to run down to 2.0% for two rings of 4 (acuens-stringens). In *Campanula* (Gairdner and Darlington 1931) a ring for four chromosomes is said to disjoin normally in two-thirds of the divisions. The proportion of non-disjunction for the larger rings of twelve or fourteen chromosomes is also about 30.0%. Sax and Anderson (1933), however, point out that the published data give an average of 41.0% of non-disjunction. In *Rhoeo* (Sax and Anderson 1933) there is about 80.0% of aneuploidy, but the rings are large (twelve chromosomes). In *Tradescantia reflexa* (Sax and Anderson 1933), rings of four gave 83.0% aneuploidy, although *Trad. edwardsiana* is of the semi-sterile type.

*These are the values to be considered (from their Table 16); for the percentage of irregularity in the distribution of the chromosomes to the poles of the spindle is one-half less, since genetic non-disjunctions in which the daughter-cells receive equal numbers of chromosomes are not counted. In certain cases the values for this latter aberration are more than the expected half of the irregularities in the zig-zag arrangement, occasionally even exceeding them. In these cases it would seem justifiable to take twice the figure in the final column as the maximum percentage of aneuploid gamete formation.

It is the investigation of the factors underlying this difference between the "semi-steriles" or *Drosophila*-like translocations, and the *Oenothera*-like type with more directed segregation, which this series of experiments has attempted to further. It should be emphasized, however, that segregation in the former type is *not* at random. That is, there is even here a preferential formation of orthoploid gametes; otherwise the ratio would not be approximately one orthoploid gamete to one aneuploid, but one orthoploid to two aneuploids. This has not always been clearly recognized. Random assortment of four objects, two by two, gives six equally likely pairs; there are four aneuploid combinations to the two orthoploid. It follows that in discussing the factors responsible for the differences between the *Drosophila*-like and the *Oenothera*-like segregation, we should bear in mind that they are more likely of degree than of kind.

Before discussing the data, a mention of various previously suggested factors may be helpful. Disjunction is normally preceded by synapsis, and it is generally supposed that the failure of synapsis would be a major factor in causing abnormal segregation. Dobzhansky (1931) formulated his hypothesis of competitive pairing primarily to account for the decrease of crossing-over observed in heterozygous translocations, but it may also be applied to the behavior of the chromosomes in segregation. Thus, a compound chromosome simultaneously attracted in different regions to two partners, might pair: (a) with both partners, followed by segregation from both; or (b) with either one alone, followed by segregation of the paired partners, the third partner moving to one pole or the other at random. On this assumption, the ratio between these types of behavior, correlated with the respective lengths of the two portions of the compound chromosome, determines the proportions of orthoploid and aneuploid gametes obtained from the individuals carrying such a complex. Inversions, from their effect in suppressing crossing-over, have also been thought to bring about a failure of synapsis (Brink and Cooper 1932; Dobzhansky 1933).

As the present paper presents further evidence bearing on the relation between crossing-over and disjunction, and on the effect of inversions, discussion of these factors will be reserved until after the presentation of the data. A third, more direct, source from which we might judge the effect of the failure of synapsis upon disjunction is to be found in the relative frequency of orthoploid and aneuploid gametes formed by rings (synapsis complete) and by chains (synapsis incomplete).

Darlington (1932) has suggested another factor which may be of major importance in determining the regularity of segregation in some forms, and its absence in others. "An association with interstitial chiasmata is relatively rigid; its shape shows little variation, and is determined by the number and distribution of the chiasmata . . . An association with terminal chiasmata is extremely pliable; the variation found in its shape shows that (as might be expected) any two chromosomes move freely in relation to one another unless they are associated at both ends . . . Forces of repulsion associated with spindle formation are therefore able to effect regular disjunction of a ring with terminal chiasmata although they are powerless to distort a configuration held together by numerous interstitial chiasmata" (pp. 142-43). This suggestion would seem, however, in need of further substantiation, as it is based on very few cases. Thus, while *Datura*, *Oenothera*, and *Campanula*, with low percentages of aneuploidy, have their chromosomes associated by terminal chiasmata at metaphase, and while *Pisum*, *Zea*, and *Tradescantia edwardsiana*, with 50% of aneuploidy, have either subterminal or interstitial chiasmata, on the other hand *Tradescantia reflexa* and *Rhoeo* (Sax and Anderson 1933) with about 80% of aneuploidy, have either partially or wholly *terminal* chiasmata.

The factors affecting segregation which are chiefly considered in the present paper are those arising from the positions of the breaks with respect to the ends of the chromosomes, and with respect to the positions of the spindle-fiber attachments.

The positions of the breaks with respect to the ends of the chromosomes affect the shape of the cross figure which is obtained whenever the chromosomes involved in a translocation are diagrammed with all homologous loci in apposition. This has been taken into account by Dobzhansky (1932) in his competitive pairing hypothesis, when he assumes that the longer portion of the compound chromosome will tend to conjugate with its homologue more frequently than will the shorter portion with its homologue; and by the author (Glass 1933). The shape of the crossfigure can vary in two fundamental ways: in the first place, the lengths of the axes may be similar or dissimilar; and, secondly, the intersection may lie at the center of both axes, of one, or of neither. The types resulting from the first sort of variation may be conveniently called equiaxial* and heteroaxial. The terms bimedral, monomedial, and amedial may

*In a preliminary abstract (Glass 1935), the term symmetrical has been used for equiaxial. Since the common meaning of symmetry would apply it to some of the heteroaxial types, this term is being discarded in favor of the less ambiguous 'equiaxial'.

be used to distinguish the types resulting from the various positions of the translocation points, that is, of the intersection of the cross-figure. By bimedral is meant the condition existing when the intersection of the cross is at the midpoint of both axes; by monomedial, when it is at the midpoint of one; by amedial, when it is at the midpoint of neither (previously called acentric).

The studies of Dobzhansky and Sturtevant (1931), based on two mutual translocations of chromosomes 2 and 3, each broken close to the spindle-fiber (i.e., in the middle), show that in the case of a bimedral cross-figure with equal axes, the aneuploid classes are approximately equal in size, and are considerably smaller than the orthoploid classes. In one of the cases, later more completely analyzed by Dobzhansky (1933), the gametic ratio is 1.0 : 0.47 : 0.43 for the respective frequencies of the orthoploid and the two types of aneuploid gametes.

In a preceding paper (Glass 1933) similar preliminary tests were made, using mutual translocations of chromosomes 2 and 3, available in connection with the dominant mosaic allelomorphs of brown at 2—104.5 (Glass 1934a). These had a different type of configuration. As 3 was broken in the middle in each case, while 2 was broken very close to the right end, the cross-figure is amedial and heteroaxial.

Also reported here are studies on the monomedial equiaxial type, formed by combining two different translocations. By comparing the results obtained from this type with those obtained for the bimedral equiaxial and the amedial heteroaxial types, it has been possible to distinguish to a certain degree the separate effects of the bimedral vs. monomedial condition, and of equality vs. inequality of the axes.

TESTS WITH AN AMEDIAL HETEROAXIAL TYPE

A. Tests with V_3

Two stocks were made up, one carrying Cy in the normal 2, C_3X in the normal 3, and V_3 , st , and Pr in the 2 and 3 carrying the translocation; the other carrying V_3 in the translocated chromosomes, and homozygous for st .* To avoid confusion of classes by crossing-over, the latter stock supplied the female parents for the pair cultures, the former the males. The cross was therefore exactly similar to that diagrammed for V_4 in Fig. 1.

The data presented in Table 1 agree with the results obtained for V_3 in a preliminary test (Glass 1933). Both reveal an anomaly which might produce considerable error in calculating the gametic

*Symbols. V_3 is Variegated-3, a dominant mosaic allelomorph of bw at 2—104.5; Cy is Curly, dominant in 2L, associated with inversions of 2L and 2R, and homozygous lethal; C_3X is an inverted, homozygous lethal, chromosome 3, produced by x-rays; st is scarlet, 3—44.0; Pr is Prickly, 3—90.0.

Table 1.—V3 st/st ♀ × V3 st Pr/Cy C3X ♂

V3 st Pr	426	
Cy	525	
V3 Cy	400	orthoploid
V3/V3 st Pr	38	
Cy V3 st Pr	151	
V3	279	aneuploid
V3 Pr	7	
V3 st	1	
	<hr/>	
	1827	
No. cultures	36	
x:y:z* = 1.0 : 0.79 : (0.12)		

*In stating the gametic ratio in the tables, we use x to represent the frequency of orthoploid gametes; y, the frequency of aneuploid gametes resulting from disjunction along one axis of the cross figure; z, the frequency of those resulting from disjunction along the other axis; and a, the frequency of those resulting from disjunction in which three chromosomes go to one pole and one to the other.

ratios, were it not taken into account; namely, one of the complementary aneuploid classes is almost twice the size of the other. This should no doubt receive an explanation similar to that given for the corresponding phenomenon in the V_4 tests, which is explained in that connection, and has been more fully discussed in an earlier paper (Glass 1934b). If, then, the size of the V_3 class in the above table be taken as typical, we find that 52.4% of the gametes formed are orthoploid; 41.4% are aneuploid gametes in which disjunction occurred along the major axis of the cross; and 6.3% are aneuploid gametes derived from disjunction along the shorter axis. If we neglect the secondary aneuploid type, as seems advisable from the small numbers of individuals, all of one class, which were obtained, we have 44.1% of aneuploid gametes to 55.9% of orthoploid gametes. The single V_3 st individual unfortunately could not be tested as to its gametic derivation, but it was probably due to a union of a spermatozoon carrying only the single, translocated 2,3 chromosome bearing st, with an egg carrying three chromosomes, namely, a normal 2, a 2,3 bearing V_3 and a normal 3 bearing st.

B. Tests with V4

Tests with stocks exactly similar to those used for V_3 were carried out in a parallel fashion. They are diagrammed in Fig. 1, and the results are given in Table 2.

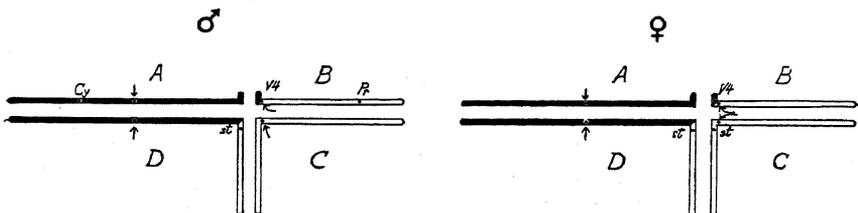


Figure 1.—T2, 3-bw V_4 st Pr/Cy C3X ♂ × T2, 3-bw V_4 st/st ♀. Chromosome 2 and portions solid; chromosome 3 and portions, in outline. Arrows designate loci of spindle-fiber attachments. A, B, C, D, the four chromosomes of the ring.

Table 2.— V_4 st/st ♀ × V_4 st Pr/Cy C3X ♂

	1	2*	3	4	7	
V_4 st Pr	930	321	212	326	1789	
Cy	970	365	263	302	1900	
Cy V_4	877	288	246	289	1700	orthoploid
V_4/V_4 st Pr	146	56	6	65	273	
Cy V_4 st Pr	333	95	128	153	709	
V_4	627	183	210	222	1242	aneuploid-1
V_4 Pr	1				1	
Cy V_4 st		1			1	aneuploid-2
st Pr	2	1	1		4	
Cy st Pr	1				1	
TOTALS	3887	1310	1066	1357	7620	
No. cultures	77	24	13	18	132	

$$\frac{1789 + 1900 + 1700}{3} = \frac{5389}{3} = 1796$$

$$x:y = \sqrt{1796} : \sqrt{1242} = 1.0 : 0.83$$

*The slight difference between the values in this column and those given for the same set of data in Glass 1934b is due to the exclusion here of four cultures containing less than twenty-five individuals each.

The striking difference in size between the two complementary aneuploid classes has been discussed in detail in Glass 1934b. From the data presented there and given in full in Table 3, it is obvious that the reciprocal cross shows no such difference. It follows, therefore, that the phenomenon is not one of differential viability of the classes; nor can it be due either to a gametic lethal, or to lethality of gametes carrying a particular type of chromosomal abnormality. The most likely explanation is that the presence of ordinary recessive lethals, by crossing-over, eliminates the non-disjunctional zygotes carrying them in homozygous condition. This interesting case, however, does not alter the validity of the data for use in our study of segregation.

Column 1 in Table 3 is a single mass culture, which is included since the ratio is the same as for the others, and the test therefore seems comparable. A difference in the food used accounts for the higher yields in the first and second experiments.

Attention should also be directed to the four *st Pr* and the one *Cy st Pr* individuals listed in Table 2; and the one *Cy st* and eleven + individuals in Table 3. In the experiments summed up in Table 2 the P_1 ♂♂ were of the constitution V_4 st Pr/Cy C3X (see Fig. 1), and the V_4 and *Pr* genes were borne in the same chromosome.* It follows that an individual bearing *Pr* and not V_4 must be due either to crossing-over in the male, or to a reversion or suppression of V_4 . As the first explanation seemed very improbable, these individuals

*This may be only apparent, as V_4 might just as well lie at the point of breakage in the other compound chromosome, 2L-3L; but as no individuals survive which do not obtain both of these loci, if they obtain either, they all therefore carry V_4 ; hence V_4 may be said to lie in the chromosome bearing *Pr*, at the point of breakage.

Table 3.—V4 st Pr/Cy C3X ♀ × V4 st/st ♂

V4 st Pr	155	(165)*	295	(312)	274	(297)	179	(189)	903	(963)	2756	
Cy	145	(158)	278	(294)	259	(283)	145	(152)	827	(887)	2787	
V4 Cy	139	(147)	305	(334)	248	(272)	177	(183)	869	(936)	2636	orthoploid
V4/V4 st Pr	66	(69)	93	(106)	43	(48)	13	(15)	215	(240)	513	
Cy st V4 Pr	86	(94)	205	(226)	143	(158)	126	(131)	560	(609)	1319	
V4	97	(111)	181	(202)	141	(164)	120	(131)	539	(608)	1850	aneuploid-1
V4 st	10		17		23		10		60			
Cy Pr	13		16		24		7		60			
Cy V4 Pr	8		29		24		6		67			crossovers
V4/V4 st	3		13		5		2		23			
Cy V4 st	8		21		15		5		49		1	
V4 Pr	9		18		20		11		58	crossovers	1	aneuploid-2
Cy st			1						1			
V4/V4 Pr	5		3		3				11		2	
TOTALS	746		1475		1222		801		4244		11864	
No. cultures	1		16		23		11					

$$\frac{963 + 887 + 936}{3} = \frac{2786}{3} = 929$$

$$\frac{609 + 608}{2} = \frac{1217}{2} = 609$$

$$x:y = \sqrt{929} : \sqrt{609} = 1.0 : 0.81$$

*The values obtained by adding the individuals in which *Pr* crossed over in the *Cy C3X/V4 st Pr* P₁ female parent, to the respective non-crossover classes are given in parentheses. These figures are the ones comparable to those of Table 2, and have been used in calculating the ratios.

†Column GT sums the totals for Tables 2 and 3.

were tested by mating *S/Cy.D/C3X* ♂♂ or ♀♀, and then testing at least five, and generally ten, of the F₁ ♂♂ to +♀♀. In every case some of these males showed the presence of the translocation. These cases are therefore due either to a reversion of *V4* to normal, or to some suppression effect. Further tests showed that *V4* tended to reappear in the offspring of these exceptional individuals, so that it is evidently a case of suppression.

The ratio derived from Table 2 for the orthoploid and the two types of aneuploid gametes is 1.0 : 0.83 : 0.02, taking the single normal-sized aneuploid class as the standard for its type. From Table 3, using complementary classes, the ratio is 1.0 : 0.81. Since these values are very close, it would seem permissible to lump the data, as is done in the last column of Table 3. The weighted ratio is 1.0 : 0.823. That is, 55% of the gametes are orthoploid; and 45% are aneuploid, arising from disjunction along the major axis. The other type of aneuploid gametes can only be calculated from the data of Table 2, where it forms 1% of the gametic total, a frequency that is so small as to be negligible in calculations based on both tables.

C. Tests with V5

Stocks similar to those used for the V_3 and V_4 tests were prepared for V_5 , and crossed in pair cultures as before. The results are given in Table 4.

Table 4.—V5 st/st ♀ × V5 st Pr/Cy C3X ♂

	<i>I</i>	<i>2</i>	<i>T</i>	
V5 st Pr	180	83	263	
Cy	185	103	288	
V5 Cy	167	80	247	
V5/V5 st Pr	38	6	44	orthoploid
Cy V5 st Pr	92	33	125	
V5	160	86	246	aneuploid-1
V5 Pr	1		1	
Cy V5 st				aneuploid-2
+	1		1	
st Pr	1		1	
TOTALS	825	391	1216	
No. cultures	8	2 (mass)		

$$x:y:z = 1.0 : 0.96 : 0.06$$

From the ratio it can be seen that the more common aneuploid type is here more frequent for V_3 or V_4 . Since V_3 , V_4 and V_5 are broken at identical points in both 2 and 3 (unpublished evidence), a ratio of approximately 1.0 : 0.82 is to be expected. The ratio observed is 1.0 : 0.96. The large difference between the ratio observed for V_5 and that for V_4 is statistically significant. Whether this is an indication of a genetic variation in the stocks used, or of other factors, cannot be said with certainty.

Tests With A Monomedial Equiaxial Type

The two mutual translocations Grape and V_4 were next combined. These both involve chromosomes 2 and 3, and differ only in that the former is broken to the right of the spindle-fiber attachment in 3, at the locus of pink (p-48.0); while the latter is broken to the left of the spindle-fiber. Each of these translocations has therefore an amedial heteroaxial figure, differing from one another as mirror images. The gametic ratio in the Grape translocation is similar to those found in V_3 , V_4 , and V_5 (Glass 1933). Both translocations possess a small chromosome, attracted to only one synaptic mate; but in V_5 this consists mainly of 3R, while in Grape it consists mainly of 3L. In the combination of the two translocations, therefore, there would be two such small chromosomes; and if it be supposed that in the previous tests the presence of such a chromosome, attracted principally to a single mate, was responsible for the almost total absence of one type of aneuploid gamete, then the presence here of two such chromosomes might lead to the elimination of both types of aneuploids, with resultant completely directed segrega-

tion. The cross-figure resulting from the combination of *V5* and Grape, diagrammed in Fig 2, is practically equiaxial, and only one axis is bisected at the intersection.

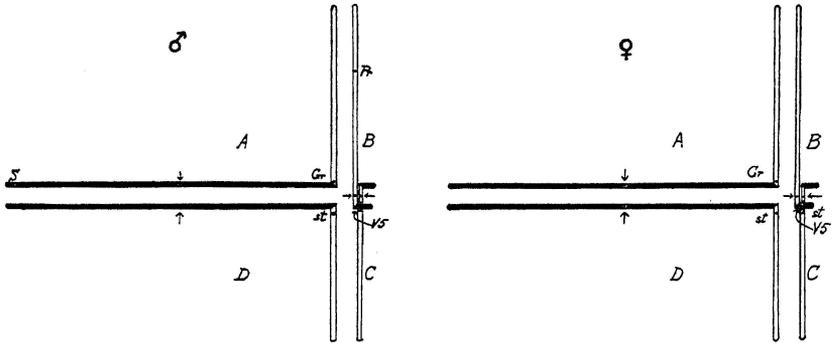


Figure 2. — T 2, 3-p^{Gr} S/T 2, 3-bw^{V5} st Pr ♂ × T 2, 3-p^{Gr} st/T 2,3-bw^{V5} st Pr ♀. Portions of chromosome 2, solid; portions of chromosome 3, in outline. Arrows designate loci of spindle-fiber attachments. A, B, C, D, the four chromosomes of the ring.

Females homozygous for *st*, and carrying both the *V5* and the *Gr* translocations were crossed to males similarly carrying both translocations, with the markers *S* (Star, 2—1.3), heterozygous *st*, and *Pr*, The double dominant eye-color with scarlet is a faintly mosaic orange. The data are given in Table 5.

Table 5.—*V5 st/st Gr ♀* × *S Gr/V5 st Pr ♂*

<i>S Gr/V5</i>	861		
<i>V5 Pr st/Gr st</i>	772		orthoploid
<i>V5 Pr st/V5 st</i>	189		
<i>S V5 Gr Pr/st st</i>	63		
<i>V5 Gr</i>	62		aneuploid-1
<i>S Gr st/V5 st</i>	96	-14 = 82	
<i>V5 Pr/Gr</i>	84	-14 = 70	aneuploid-2
<i>S Gr V5 Pr</i>	16		
<i>V5 st Gr/st</i>	13		3/1-aneuploid
<i>S st Pr</i>	1		
<i>st Pr</i>	1		
TOTAL	2159		
No. cultures	32		

$$\frac{861 + 772}{2} = 816$$

$$\frac{63 + 62}{2} = 62.5$$

$$\frac{82 + 70}{2} = 76$$

$$x:y:z = \sqrt{816} : \sqrt{62} : \sqrt{76} = 1.0 : 0.27 : 0.30$$

$$x:a = \sqrt{816} : \sqrt{14.5} = 1.0 : 0.13$$

The ratio shows that while the two aneuploid types have decreased from 45% to 31% in comparison with V_3 , V_4 , and V_5 and Gr , yet the condition is far short of being completely directed segregation. For while the aneuploid type found in V_3 , etc., has been reduced from 45.0% to 14.8%, the other aneuploid type, practically absent in V_3 , etc., amounts to 16.4%. The 2-chromosome aneuploid types are equal, as in Dobzhansky's case (1933). A group of unexpected individuals is also represented by the $S Gr V_5 Pr$ and $V_5 Gr st/st$ phenotypes. These can only be due to the union of a 3-chromosome gamete from one parent with a complementary 1-chromosome gamete from the other. The markers used will suffice, however, to detect only one of the four possible pairs of such complementary gametes. The other three would resemble in phenotype either the main orthoploid classes, or the first or second group of aneuploid classes. But if, as seems probable, the appearance of these 3/1-chromosome aneuploids is due to the elimination of tendencies to disjoin between the small chromosomes B and C, it is likely that only two of these four types occur, namely, the ones detected, ABC and D, and a similar pair BCD and A. The average frequency, to the nearest whole number, of the type ABC and D which is represented, has been subtracted from the frequencies of the classes with which the BCD and A type are merged. Since this doubles the number of 3/1-chromosome aneuploids, the total for these is 0.26 to each orthoploid; and the ratio of orthoploid to all aneuploids is 1.0 : 0.83.

THE EFFECTS OF THE RELATIVE LENGTHS OF THE AXES UPON SEGREGATION

If we now compare the results obtained in the various types, it becomes apparent that equality of the aneuploid types is regularly associated with equality of the axes of the cross-figure. In Dobzhansky's case (Dobzhansky 1933) with equal axes and bimedral intersection, the two aneuploid types coming from segregation along the two axes of the cross-figure are equal. In V_3 , V_4 , and V_5 , as well as in Gr , (Glass 1933), with unequal axes and amedial intersection, they are very unequal. In V_5/Gr , with the intersection removed from the medial point of the longer axis just as far as in V_3 , V_4 , etc., but with equality of the axes restored, the aneuploid types are again equal.

Further evidence is available from a case reported in *Zea mays* by McClintock (1934). She studied a reciprocal translocation between chromosomes 6 and 9, in connection with the relation of nucleoli to chromosomes. In her Table 1, considering both rings and chains, there

are 345 alternate (i.e., orthoploid) distributions to 383 adjacent (i.e., aneuploid) distributions, with 170 uncertain cases. Most of the latter McClintock attributes to the difficulty of detecting alternate distributions in rings. She therefore believes the proportion of alternate to adjacent distributions in chains alone affords a more accurate measure. This ratio (216 : 199) is 1.0 : 0.92. McClintock states that in the adjacent distributions "in nearly all cases, the chromosomes were arranged on the plate so that the two chromosomes with unasociated ends were passing to the same pole". This separation is between the satellited ends of chromosomes 6 and 9⁶, so that 6 and 9⁶ go to one pole and 9 and 6⁹ go to the other. The plane of separation extends along the axis between 6⁹ and 6, on the one side, and 9 and 9⁶, on the other. From McClintock's Fig. 13, in which the two arms of this axis may be distinguished by the fact that they carry the two regions of spindle-fiber attachment, it may be seen that this axis is about three times the length of the other. Segregation occurs only along the long axis, except in rare cases; and only one type of aneuploid gamete is formed. It seems probable, although the evidence is not conclusive, that in other maize "semi-steriles" both types of aneuploid gametes are formed (McClintock 1930; Burnham 1934). If this is so, we have here an almost exact duplication of the situation in *V*₃, *V*₄, *V*₅, and *Gr*, with perfectly parallel results. With inequality of the axes we obtain predominantly segregation along the greater axis; but the orthoploid : aneuploid ratio is not materially altered.

THE EFFECTS OF CHANGE IN THE POSITION OF THE INTERSECTION UPON SEGREGATION

Upon this point the cases studied are as yet too few for the deduction of any valid general principle. Certain striking facts to be observed in these cases should be pointed out, however. The bimedi-ally intersecting type studied by Dobzhansky (1933) may be compared with the monomedially intersecting *V*₅/*Gr*. In the former, there were 52.6% of orthoploid gametes, and 47.3% of aneuploids, the two types of the latter being practically equal to one another (25.0% and 22.3% respectively). This orthoploid : aneuploid ratio differs significantly from a 1 : 1 ratio. In *V*₅/*Gr*, there were 54.6% of orthoploid gametes, 14.8% and 16.4% of the two 2-chromosome aneuploids respectively, and 14.2% of the 3/1-chromosome aneuploids, making 45.4% of aneuploidy in all. The 2-chromosome aneuploids show a reduction from about one-half of all the gametes to approximately one-third. On the other hand, the appearance of 3/1-chromo-

some aneuploid gametes holds the total amount of aneuploidy at nearly the same level.

In the case of the amedially intersecting type the additional factor of unequal axes is also operating. If we eliminate its effect in causing inequality of the two aneuploid types, there remains for consideration only the slight reduction in aneuploidy observed when comparison is made with the bimedially intersecting type studied by Dobzhansky. This amounts to only 2.3% (47.3%—45.0%); or, if we assume that a perfectly symmetrical cross would give exactly 50.0% orthoploidy, the reduction is only 5.0%. These diminutions of the aneuploidy, although small, are all statistically significant. There is a consistent tendency for the orthoploid gametes to exceed 50.0%, and this tendency seems to be most marked in those types in which the intersection is removed from the midpoint of one or both of the axes. However, this interpretation must be accepted with caution, since the deviation of the V_5 ratio (Table 4) from that expected shows that large and significant variations in the amount of aneuploidy may arise from unknown causes. A possible cause for this tendency may be the fact that removal of the intersection from the midpoints of the axes results in one or more chromosomes which tend to pair with one partner only (chromosome B, Fig 1; chromosomes B and C, Fig. 2). This results in a less random distribution of the chromosomes, as is discussed more fully in at later section. If the short chromosome lies with its tiny segment on the shorter axis, as in V_3 , V_4 , and V_5 , so that the inequality of the axes tends to make it segregate from its sole partner anyway, this tendency to non-random segregation is masked. On the other hand, when it lies on an axis equal to, or longer than, the other, as in V_5/Gr , this effect is more apparent, and the 2-chromosome aneuploids are reduced as much as 20.0%.

THE EFFECT OF SPINDLE-FIBER POSITION ON SEGREGATION

Darlington (1932, 1934) maintains that the regularity of segregation is determined, not by the repulsion of homologous loci, subsequent to pairing, but by the repulsion of spindle-fiber attachments. This implies, however, the prior occurrence of regular synapsis, since the repulsion of two adjacent (non-homologous) spindle-fiber attachments would certainly not result in regular orthoploid disjunction. Sax and Anderson (1933) has pointed out that *Datura*, *Oenothera*, *Campanula*, and *Tradescantia occidentalis*, all forms with low aneuploidy, have median spindle-fiber attachments; while *Zea*, *Pisum*, *Trad. reflexa* and *Trad. edwardsiana*, and *Rhoeo*, all forms with fifty per cent ster-

ility or higher in rings, have subterminal or submedian attachments. However, Dobzhansky and Sturtevant (1931) and Dobzhansky (1933) have shown conclusively for *Drosophila* that there is no homology of spindle-fiber attachments. At least, other things being equal, "homologous" attachments undergo non-disjunction as frequently as "non-homologous", and this in a translocation with medially attached spindle-fibers. Further, in the tests reported here for V_3 , V_4 , and V_5 , in which one pair of the spindle-fiber attachments is located at a distance from the intersection, there is only a slight alteration in the aneuploid frequency. It has been suggested to the author by several workers that the Dobzhansky case is not conclusive on this point, since the spindle-fibers are all at the intersection of the cross, and therefore all four are approximately equally adjacent. Conclusive proof on this point is found in the V_5/Gr case, in which one pair of spindle-fibers, attached far from the intersection of the cross, nevertheless underwent non-disjunction as frequently as they disjoined; in fact (compare the aneuploid-2 group in Table 5 with the aneuploid-1 group), even somewhat more frequently.

THE EFFECT OF INVERSIONS UPON SEGREGATION

Dobzhansky (1933) inserted inverted sections into the arms of the type he studied (bimedial equiaxial), and from the results concludes that "the presence of an inverted section in one of the chromosomes involved in the translocation decreases the frequency of pairing of this chromosome with its homolog, suppresses crossing-over, and increases the frequency of non-disjunction of this chromosome". (p. 300) In the summary he says, "Inverted sections . . . cause a more or less random distribution of these chromosomes at the reduction division"; and "the presence of inverted sections in some of the chromosomes . . . decreases the frequency of non-disjunction of other chromosomes involved in the translocation". (p. 304). But examination of his data shows that this effect was observed only when both of the two inversions used lay in the two arms of the same axis. When two inversions were inserted in opposing axes, the result was not very different from that observed in the absence of inversions. While it is true in a general sense that inverted sections produce a more or less random distribution of the chromosomes, the effect in translocations tends toward a less random distribution. One aneuploid type is eliminated, and the orthoploid frequency is increased.

Now it might be supposed that, should four inversions be used, covering all four arms of the figure, the result might be an intensification of either the effect of a chromosome bearing inversions in each arm; or of the effect of an axis bearing inversions in each arm. In

the former case, there would be little or no apparent effect on segregation. In the latter case, both types of aneuploid disjunction would be practically eliminated, and we would expect almost completely directed, orthoploid segregation, comparable to that found in rings of *Datura* and *Oenothera*. It has indeed been suggested, by Brink and Cooper (1932), that inversions may be the cause of the regular disjunction in these forms. This point of view has been criticized by Sterling Emerson (1932), although at the time decisive data were not on hand. Indeed, Dobzhansky's later finding that inversions may reduce irregularity in disjunction would seem to nullify Emerson's claim that their presence would cause a more random segregation.

In the tests reported for V_3 , V_4 , and V_5 in this paper the second chromosome of the male carried the two *Cy* inversions, and the inversion (or inversions) known as C_3X similarly covered practically the entire extent of the third chromosome; while the female was free from inversions. In the second series of experiments on V_4 (Table 3), the inversions were carried in the female and not in the male. These inversions, together with the effect of breakage in 2R, cover the four arms of the figure. The results show that the difference in the ratios obtained when the inversions are carried in the $\sigma^7\sigma^7$, and when carried in the $\text{♀}\text{♀}$, is very slight. Further, in the preliminary experiments reported by Glass (1933), when there were no inversions present in either male or female, practically the same ratios were obtained (1.0 : 0.71 for V_3 ; 1.0 : 0.72 for V_4 ; 1.0 : 0.66 for V_5 ; 1.0 : 0.67 for *Gr*). These data, showing the absence of effect when inversions are present in all arms, indicate the limited application of Dobzhansky's conclusions. Only when inversions are working together (i.e., lie on the same axis), and are unopposed by the effects of inversions lying on the other axis, do they exert an influence on segregation. It would be interesting to see what effect a single inversion would have, as well as the effect of two inversions, lying on one axis, opposed to one on the other, in a symmetrical cross. The V_4 case is somewhat similar to the latter, lacking only the symmetry, for the long axis carries both *Cy* inversions plus the right part of the C_3X inversion, while the short arm has only the left part of the C_3X inversion.

It should also be noted that even in the experiment in which Dobzhansky had two inversions in the two arms of the same axis, and none in the other axis, the reduction of aneuploidy amounted to only 5.2%. He obtained (present author's calculations*) 57.8% of

*Calculation: *Cy Bl* = 4324, including two orthoploid classes and one class of each aneuploid type. Subtract from this 773 + 8, the frequencies of the known complementary aneuploid classes, divide by 2, and then find the gametic ratio by taking the square root of the result, of 773, and of 8.

orthoploid gametes and 38.2% and 4.0% of the two types of aneuploid gametes respectively. The maximum reduction of aneuploidy through the presence of inversions in a ring of four can hardly be more than about 8.0%. This decrease is nevertheless statistically significant.

SEGREGATION IN RINGS COMPARED WITH THAT IN CHAINS

Sax and Anderson (1933) find that disjunction is more regular in chains than in rings, in both *Rhoeo* and *Trad. edwardsiana*. They suggest that Gairdner and Darlington (1931) may have been in error in reporting the opposite situation as prevailing in *Campanula*, since they fail to figure certain types (S-shaped chains) which, though classed as non-disjunctional, may very well have been disjunctional. If this were true, non-disjunction would be greater in the rings than in the chains. But there seems to be no reason to suppose that this situation exists in all forms. A survey of the *Oenothera* types (Cleland and Oehlkers 1930) for which the data are given (*velans-gaudens*; *flavens-stringens*; *albicans-strigens*; *deprimens-flavens*; *deprimens-gaudens*; *deprimens-velans*; and *gaudens-stringens*) shows that rings disjoin more regularly than do the chains, in all cases except the last.

McClintock (1934) presents in her Table 2 further data on segregation in a series of crosses where chromosome 6 carried an abnormally large satellite. Although this had a pronounced effect on the relative proportions of rings and chains, raising the latter from 50% to 83% of the total, there is no effect upon the frequency of aneuploidy. The orthoploid :aneuploid ratio (414 :401) remains 1.0 : 0.97. Factors affecting the relative frequencies of rings and chains do not necessarily affect the type of segregation.

THE RELATION BETWEEN CROSSING-OVER, SYNAPSIS, AND SEGREGATION

Darlington (1932, 1934) has advanced the theory that all chiasmata result from crossing-over, and that metaphase association of homologues is the result of this chiasmatisation. He further supposes that segregation at anaphase is due to the repulsion between spindle-fibers, which overcomes the waning attraction between homologous loci. This may indeed be the immediate cause of the separation of the chromosomes; but, as we have seen in a previous section, it cannot be the mechanism whereby the segregation is directed. It is more generally supposed, as is indicated in the introduction to this paper, that the metaphase association of homologues is the directive

factor; and perhaps Darlington would agree to this. But it is also held that metaphase association, due to chiasmata in some cases terminalized, in others remaining in interstitial form, is a consequence of the closer prophase association during which crossing-over occurs. Probably few would question that a close prophase pairing of homologues is an essential prerequisite to crossing-over.

However, the data now at hand seem to point to the conclusion that there is no such direct relationship between crossing-over and segregation as this implies. Dobzhansky (1933) showed that non-disjunction of the arms of the autosomes of *Drosophila* involved in a reciprocal 2,3 translocation reduces crossing-over; while in the arms disjoining normally, crossing-over is increased. An examination of the data in his tables 5 and 9 (l.c.) shows that the reductions are only 8.15 units in 2L; 12.02 in 2R; 8.68 in 3L; and 18.0 in 3R—an average of about 26%. Thus, crossing-over in arms undergoing non-disjunction is about 75% of the normal value; and hence, if every chiasma results from a cross-over, there are 75% of the normal number of chiasmata in the arms undergoing non-disjunction.

In a recent paper, Gershenson (1935) studied primary and secondary non-disjunction in *Drosophila* ♀♀ carrying a C1B (inverted) X-chromosome. The ordinary rate of secondary non-disjunction, as shown by XXY sisters, is only 3.9%; but for the XX(C1B)Y ♀♀ it is 35.7%. This extraordinarily high value can be shown to be due to the effect of the inversion rather than to any specific genes in the C1B chromosome. Nevertheless, crossing-over in XX(C1B)Y ♀♀ is equal to that in their XX(C1B) sisters, in spite of the difference of 35.36% of non-disjunction. Again, in primary non-disjunction, XX(C1B) ♀♀, in which crossing-over is practically eliminated, would be expected on Darlington's hypothesis to yield a high percentage of non-disjunction. Instead, it is only 0.34%, about 1/100 of the amount to be expected according to the hypothesis.

The data presented in this paper in the section on inversions also show that complete absence of crossing-over is not associated with any material alteration in the orthoploid:aneuploid ratio. It is true that the relative proportions with which the various aneuploid types are formed may be affected. Translocation females carrying inversions along one axis have both crossing-over and segregation along that axis completely inhibited. But such females with inversions along both axes, while they have crossing-over completely inhibited for both axes, have a type of segregation just like that of similar individuals carrying no inversions. This is similar to the lack of effect of

these same inversions upon segregation in non-translocation females, in which they also inhibit crossing-over. Dobzhansky (1933) found that even in males, where crossing-over is normally absent, inversions placed along one axis of a translocation had a small effect upon the relative sizes of the two aneuploid types. Obviously, chromosomes may disjoin quite regularly without having crossed over, or may fail to disjoin with crossing-over only slightly reduced; in spite of a general tendency for the two to vary together.

Dobzhansky (1933) concluded that "the occurrence of crossing-over does not determine directly the course taken by the chromosomes at disjunction. Both crossing-over and disjunction are determined by a third factor, which is, apparently, the pairing of the chromosomes at the stages preceding the occurrence of crossing-over." (p 304). Nor is synapsis the sole prerequisite of both crossing-over and segregation. If the close pairing of homologous loci is the cause for their regular disjunction, then this state may be a necessary precursor to crossing-over; but other important factors must later enter in to determine whether or not crossing-over will occur. On the other hand, if crossing-over regularly occurs between synapsed strands, then synapsis, in the sense of close pairing, cannot be the immediate cause of segregation. Mavor (1929) found that x-rays affect non-disjunction in the oldest eggs treated, and that crossing-over is affected only in eggs laid four days or more after treatment. This indicates that crossing-over precedes the determination of disjunction, and makes the second alternative more likely. Winchester (Patterson, Brewster, and Winchester 1932) found that non-disjunction following x-ray treatment was highest in relatively mature eggs, and fell to a constant level, still significantly higher than in the controls, after the sixth day. This increase in non-disjunction which occurs at the same time that crossing-over is being affected may therefore be due to the effect of x-rays upon synapsis; but the greater effect must be produced later in the course of events leading to disjunction. These considerations make intelligible the frequently observed correspondence between a diminution of crossing-over and an increase in non-disjunction, such as Anderson (1929) found in the X-chromosome. At the same time, since one process need not inevitably accompany the other, conditions where there is a failure of correspondence, such as those discussed here, may also be satisfactorily interpreted.

If orthoploid segregation regularly follows metaphase association of homologues, and if this metaphase association is brought about physically through chiasmata, then chiasmata cannot be the simple results of crossing-over. The most plausible conclusion would appear to be that, as

Sax has claimed, chiasmata are not the results of crossing-over, but are the precursors of crossing-over, with no essential numerical relation of one chiasma to one cross-over (Sax 1930). However, in view of the evidence previously discussed, showing that in *Zea* segregation in rings and chains is similar, although in the latter we have an evident failure of association at one point, the relation of metaphase association to segregation appears uncertain.

These considerations, of course, do not affect the validity of the suggestion made by Darlington (1932) and discussed in the introduction, that the reason for the greater degree of orthoploidy in some ring-forming species than in others lies in the greater flexibility of terminal chiasmata as compared with interstitial. Emsweller and Jones (1935) have just published a preliminary account of a case occurring in *Allium* in which different stocks manifest either interstitial or terminal chiasmata as though the type of chiasma were controlled by the presence of a single recessive gene for interstitial chiasmata. The most fertile plants, as measured by the number of seeds produced per umbel, all had the interstitial type of chiasmata. Such a gene, unfavorable in ordinary races, might be decidedly beneficial in ring-forming types; and selection would be expected to eliminate those ring-forming types which did not carry it, with their lower percentage of orthoploidy.

THE NON-RANDOM NATURE OF SEGREGATION

It has been pointed out in the introduction that random segregation of four chromosomes in a ring would give equal numbers of six sorts of gametes, two orthoploid, two aneuploid resulting from segregation along one axis, and two aneuploid resulting from segregation along the other axis. This would be expected to yield 33.3% orthoploidy. The proportion of orthoploids in rings in *Drosophila*, *Zea*, *Pisum*, and similar forms fluctuates, however, not around this value, but around 50.0%. Burnham (1934) states that "absolutely random 2 by 2 distribution in the ring of four should give 50 per cent of spore abortion; since for each of the two 'open' orientations, there should be a corresponding zigzag arrangement occurring with equal frequency". This statement may be taken to imply that the 50.0% observed orthoploid frequency is due to the fact that such a ring, opened out in metaphase, may orient itself so that segregation will take place along either axis. Considering these separately, each will give four gametes by random assortment two-by-two, of which two will be orthoploid and two will be aneuploid. Adding together the results for the two axes, one would arrive at 50.0% orthoploidy. This seems very plausible, if we may assume that the segregation can be considered along the

two axes independently. We may denote the four chromosomes in a ring of four consecutively as A, B, C, and D. Then when A separates from B, C will separate from D; and further, as D is separating from C, it is not at all influenced by A, since it goes at random either with A or away from A. This means, in essence, that no chromosome in the ring is simultaneously under the influence of two partners, as far as segregation is concerned, while, at the same time, it may cross over with both (Dobzhansky 1933). If a chromosome can be influenced by two partners at the same time, the orientation of C and D with respect to that of A and B would not be at random; but instead alternates in the ring would go more frequently to the same pole. This would produce a higher frequency of orthoploids than of aneuploids.

In this connection the *V5/Gr* case is illuminating. Here, with equal axes and therefore equal aneuploid types, segregation is distinctly non-random, even with the supposition of the prior determination of one axis of segregation or the other. For, if segregation occurs at random, after the axis is determined, then, whenever segregation took place along the vertical axis (referring to Fig. 2), one should get equal numbers of orthoploid gametes and of 2-chromosome aneuploid gametes of one type; whenever segregation took place along the horizontal axis, one should get equal frequencies of orthoploid and of 2-chromosome aneuploid gametes of the other type, with double the frequency of 3/1-chromosome aneuploids, of two types. Instead, the 3/1-chromosome aneuploids merely equal in frequency the 2-chromosome aneuploids of the same group; while, since aneuploids of both types together amount to only half of the orthoploids, presumably each aneuploid type is only half of the orthoploids coming from the same axial segregation. That is, instead of an expected ratio of 1 : 1 : 2, a ratio of 2 : 1 : 1 is obtained. It must be concluded that, for this case, it is not legitimate to postulate segregation as proceeding by first, a determination of an axis of segregation; and second, random distribution. Nevertheless, the small chromosomes B and C have so small a paired portion that they may be regarded as being unaffected by competitive pairing; that is, B should always pair with A, and C with D. One would then expect that when A paired with D, the only gametes obtained would be AC and BD. But the results show that B disjoined from A only five-eighths of the time when A disjoined from D, for the frequencies of gametes AC plus A are .63 to .37 for AB plus ABC; and the same is true for C with respect to D. This is a difference of only one-eighth from random segregation of B or C when A and D disjoin, while the absence of the tendency on

the part of these small chromosomes B and C to disjoin from one another would lead us to expect a complete departure from such a random segregation. In other words, disjunction of A from D must tend to inhibit the disjunction of A from B. Similarly it may be shown that disjunction of A from B tends to inhibit disjunction of A from D. This clearly shows that a chromosome tends to disjoin from only one mate at a time. It follows that segregation must usually occur along a single predetermined axis. The non-random distribution observed in the present case, one-eighth of the time, must be due to the few instances in which one chromosome did disjoin from two mates simultaneously. It is no doubt attributable to the absence of any tendency to disjoin on the part of B and C, a lack confirmed by the frequent occurrence of the 3/1-chromosome aneuploids.

GENERAL CONCLUSIONS

These multifarious data may be summed up most advantageously in the form of a general hypothesis of segregation in rings of four. Some of the postulates are well established by evidence now available; others are based on single cases, and are in need of confirmation, but will serve as a guide to further experimentation.

1. Segregation in general is determined by the forces exerted between homologous loci.

This postulate depends upon the proof that the spindle-fiber attachments have no greater force in determining the direction of segregation than other loci; and upon the evidence showing that equal axes produce an equality in the types of segregation, while factors such as diminution of competitive pairing, inversions, etc., which may be expected to alter the degree or direction of the forces exerted between the homologous loci, affect segregation correspondingly.

2. Segregation in a 4-chromosome ring tends to take place along a single predetermined axis so as to result in the equal numerical division of the chromosomes.

This postulate, together with the fourth, explains why disjunction in rings is non-random, and why it generally tends to average 50.0% orthoploidy to 50.0% aneuploidy. It is especially supported by the analysis of the *V5/Gr* case, in which it is shown that disjunction of a chromosome from one partial homologue tends to inhibit its disjunction from a second partial homologue which is not under the influence of competitive attraction. Bridges and Anderson (1925) showed that in triploids 2X,1A and 1X,2A gametes were seven to nine times as

frequent as 1X,1A and 2X,2A gametes. Recently, Beadle (1935) has shown that in addition $\widehat{XXX},1A$ gametes are about 48 times as numerous as $\widehat{XXX},2A$; and that noX,2A gametes are about 24 times as numerous as noX,1A. There is thus a definite tendency in *Drosophila* toward formation of gametes containing equal numbers of chromosomes, when random distribution would tend to produce more unbalanced types. It seems probable that segregation along definite axes in these translocations is an expression of this tendency; so that, regardless of aneuploidy, 2-chromosome gametes tend to be formed. This would explain why, in *V5/Gr*, the frequency of the 3/1-chromosome gametes is only one-half of the frequency expected if B and C go at random to either pole whenever A disjoins from D. It would follow that in a 6-chromosome ring the tendency to form balanced (3-chromosome) gametes could not be described as segregation along an axis.

3. Segregation along a given axis is proportional to its relative paired length.

This postulate seems firmly established on the basis of the evidence that equal axes produce equal frequencies of the aneuploid types, while unequal axes produce unequal aneuploid types. It should be noted that the effect of a paired portion less than one-third of the length of the opposing axis is negligible in the determination of the axis of segregation. The qualifying term 'paired' is necessary because of the effect of inversions inserted along one axis in eliminating segregation along that axis. Since in cases carrying inversions along both axes, the equality of the paired portions of the axes is restored, it is clear why these should show segregation like that found in the absence of inversions.

4. Except for the determination of the axis, the distribution of the chromosomes tends to be at random.

This postulate is necessary to account for the commonly observed orthoploid : aneuploid ratio of 1 : 1, as indicated above. In conjunction with the second postulate, it also explains why determination of segregation along one axis only does not alter the orthoploid : aneuploid ratio. The *V5/Gr* case, as well as all others studied, supports this interpretation.

5. A reduction of competitive pairing on the part of two of the four chromosomes in the ring leads to less random segregation.

This postulate is based solely on the *V5/Gr* case, where the acentricity of the intersection of the cross-figure gives rise to the small chromosomes with very short common homologues. It is therefore

perhaps not of general application; but a priori reasons make it plausible. However, with the decrease of random assortment observed, the diminution of competitive pairing also led to a large increase in the 3/1-chromosome gametes; so that the total orthoploidy is not materially increased by this factor.

6. The relation of crossing-over to disjunction is of a secondary nature.

This postulate seems well supported on the basis of the considerations discussed in the section on the relation of crossing-over, synapsis, and segregation. It also follows from the preceding postulates. For if the primary factor in segregation is the determination of a given axis of segregation, the degree of association and crossing-over in the other axis is immaterial. The fact that crossing-over is lowered in the arms undergoing non-disjunction may mean one of two things. If the determination of the axis of segregation precedes crossing-over, it means that the inhibition of disjunction along the other axis affects to a moderate degree the ability of crossing-over to occur along that axis. But if crossing-over precedes the determination of the axis, it would mean that, when the axes were equal in length, the one undergoing the higher frequency of crossing-over in each instance would determine the axis of segregation. This is capable of test, and should throw light on the time relations of crossing-over and the determination of segregation.

None of these postulates makes any clearer the type of directed segregation observed in *Datura*, *Oenothera*, and similar forms. Nor is there any prospect of getting clear genetic data from them comparable to that obtainable in *Drosophila*, on account of the lethality of aneuploid gametes. Darlington's suggestion that the type of chiasmata persisting at metaphase may be responsible can only be tested by assembling data as to the constant association of terminal chiasmata with low aneuploidy in a large number of ring-forming species. One other possibility arises, however, from the considerations made in this paper. Any factor which would tend to limit or inhibit the determination of segregation along a single axis, thus allowing a chromosome to be simultaneously affected in segregation by both homologues in the ring, would increase the orthoploidy.

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