FACTORS AFFECTING RECOGNITION AND DISJUNCTION OF
CHROMOSOMES AT DISTRIBUTIVE PAIRING IN
FEMALE *DROSOPHILA MELANOGASTER*.

I. TOTAL LENGTH VS. ARM LENGTH^{1,2}

CHARLEEN M. MOORE³ AND RHODA F. GRELL

*Department of Zoology and Entomology, University of Tennessee,
Knoxville, Tennessee 37916*

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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ABSTRACT

The behavior of a compound metacentric fourth chromosome ($\overline{44}$) has been examined to determine whether arm length or total length is the basis for recognition in distributive pairing. Recognition was judged by the frequency with which the $\overline{44}$ nondisjoined from a series of X duplications (Dp), ranging in size from ≤ 0.3 to > 4 times the size of a single fourth chromosome. Dp , $\overline{44}$ nondisjunction was measured in the absence and in the presence of a competitor, a compound metacentric X . In both situations, total length and not arm length, was found to confer the characteristic recognition property to the $\overline{44}$. A comparison of Dp , $\overline{44}$ nondisjunction curves for both the noncompetitive and competitive situations with analogous Dp , 4 curves previously obtained, show the Dp , $\overline{44}$ curves to be similar in shape to those obtained earlier but displaced one unit to the right, corresponding precisely to the difference in size between the $\overline{44}$ and the 4 . Rules governing chromosome recognition for acrocentrics were found completely applicable to metacentrics; disjunctive behavior of metacentrics differed from that of acrocentrics in that two arms conferred on a chromosome the capacity to act as the intermediate of a trivalent when size no longer warranted this attribute. This capacity, itself, is size-dependent.

DISTRIBUTIVE pairing in the *Drosophila melanogaster* female is recognized as that phase of the meiotic cycle during which chromosomes that had previously failed to undergo exchange can enter into segregational associations with other noncrossover chromosomes, homologous or nonhomologous. Evidence for a second type of pairing comes from the finding that, while only nonrecombinant chromosomes participate in nonhomologous associations, crossing-over between homologues is unaffected by the segregational involvement of one member with a nonhomologue (GRELL 1962).

Chromosomes available for distributive pairing are considered to make up a

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² Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Tennessee.

³ Present address: Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

pool within which a solitary member will necessarily remain unassociated and segregate randomly, two members (whether homologues or not) will pair and segregate with a high frequency, and three or more members will compete for associations and exhibit distinct preferences (GRELL and GRELL 1960). In the case of three members, the basis for preference has been shown to be chromosome size and not homology (GRELL 1964a, 1964b, 1967), in contrast to the type of pairing that precedes exchange where homology is the prime determinant. When the number of members exceeds three, the variety of pairing configurations and disjunctive patterns prohibits simple analysis.

From previous studies, rules governing the behavior of chromosomes in the pool have been formulated. In a noncompetitive situation with only two members, nonhomologues of widely different size will pair and segregate frequently; but with increasing similarity in size their segregation will approach the regularity of homologues. In a competitive situation, the ratio of sizes determines pairing and segregation behavior. With two similar and one dissimilar chromosome, the similar chromosomes pair and disjoin regularly while the third moves randomly. With three dissimilar chromosomes the intermediate tends to move to one pole and the small and large to the other pole, indicating directed segregation from a trivalent. The highest frequency of trivalents seems to occur when the intermediate element is the geometric mean of the other two.

These rules were derived primarily from the behavior of acrocentric chromosomes. The present study examines the behavior of a metacentric chromosome to determine whether size recognition in the distributive pool is a function of total length or arm length, and whether the rules derived for acrocentrics are applicable to metacentrics. The results demonstrate that total length rather than arm length controls recognition at distributive pairing. Further, while metacentrics possess the same recognition properties as acrocentrics, there is an important difference in their disjunctive behavior. This difference permits a distinction to be drawn between the recognition properties and the disjunctive properties of chromosomes during the distributive phase.

MATERIALS AND METHODS

Materials: Several free *X* duplications [*Dp*(1;*f*)], represented here by the symbol *Dp*, were chosen for the present study. The genetic content of each of the *Dp*'s is given in Table 1. Each *Dp* carries the distal tip of the *X* with the locus for γ^+ and various amounts of proximal *X* heterochromatin. Their sizes, determined at mitotic metaphase, arbitrarily giving the normal fourth chromosome a value of 1.0, range from ≤ 0.3 to > 4 and are given in Table 1 along with the origin of the *Dp*—irradiation of *sc*⁸ or Canton-S (C-S) *X* chromosomes.

The other chromosomes that were used and their symbols are: *C*(1)*RM*, a metacentric compound chromosome composed of two *X*'s attached in reverse order to a single centromere ($\overline{XX}$); *C*(4)*RM*, a metacentric compound chromosome composed of two right arms of chromosome 4 attached in reverse order to a single centromere ($\overline{44}$); and two marked *Y* chromosomes (γ^+Y and *B*^S*Y*). The lengths assigned to these, with the length of a normal 4 taken as 1.0, are: $\overline{44}$, 2.0; $\overline{XX}$, ~ 20 ; γ^+Y , ~ 9 ; and *B*^S*Y*, ~ 8 . The length of $\overline{XX}$ was derived using the measurements of *X* made by COOPER (1959) and for the γ^+Y and *B*^S*Y* from photographs of squashes of oogonia and ganglia made by the present authors. The values for $\overline{XX}$ and the *Y*'s are only approximate and should not be taken as exact. It is certain only that the γ^+Y is a little longer than the

TABLE 1
Nature of X duplications

Chromosome	Origin	Length at mitotic metaphase	X loci present							
			γ^+	ac^+	sc^+	$su(w^a)^+$	dor^+	pn^+	$su(f)^+$	bb^+ NO
Chromosome 4		1.0								
<i>Dp 1187</i>	sc^8	≤ 0.3	+	+	—	—	—	—	—	—
<i>Dp 1205</i>	sc^8	0.7	+	—	—	—	—	—	—	—
<i>Dp 1144</i>	C-S	1.1	+	+	—	—	—	—	—	—
<i>Dp 1337</i>	C-S	1.4	+	+	+	+	+	—	—	—
<i>Dp 1346</i>	C-S	2.0	+	+	+	—	—	—	—	—
<i>Dp 1488</i>	C-S	2.5	+	+	+	+	—	—	—	+
<i>Dp 856</i>	C-S	3.0	+	+	+	—	—	—	—	+
<i>Dp 1173</i>	C-S	3.6	+	+	—	—	—	—	—	+
<i>Dp 3</i>	C-S	>4	+	+	+	—	—	—	+	+

BSY and that the $\overline{XX}$ is about twice the size of a normal X . The $\overline{44}$ length of 2.0 was obtained by measuring many squashes of oogonia and ganglia of the $\overline{44}$ with the Dp 's and is more exact than the lengths given for $\overline{XX}$ and the Y 's.

The markers in this study, with their symbols and genetic locations, include: yellow² (γ^2 , 1-0.0), scute (sc , 1-0.0), white-apricot (w^a , 1-1.5), vermilion (v , 1-33.0), cubitus interruptus-Dominant (ci^D , 4-0.0), cubitus interruptus (ci , 4-0.0), and eyeless Russian (ey^R , 4-2.0). Further descriptions of these chromosomes and mutants are given in LINDSLEY and GRELL (1968).

Noncompetitive situation: Following the experimental design of GRELL (1964a) two situations were investigated, one noncompetitive and one competitive. The noncompetitive situation was set up in such a way that the distributive pool would consist of only two chromosomes. Three types of females produced this situation: (1) those with a Dp and $\overline{44}$ in the distributive pool, (2) those with a γ^+Y and $\overline{44}$, and (3) those with a $\overline{XX}$ and $\overline{44}$ in the pool. The first and second types of females were produced by introducing each Dp or γ^+Y into females carrying a $\overline{44}$ with heterozygous recessive markers (thus giving a wild-type phenotype) and two normal X chromosomes, each marked with γ^2 . The third type of female was obtained by replacing the normal X chromosomes of a female carrying the same $\overline{44}$ with a $\overline{XX}$ marked with $\gamma^2 sc w^a v$. The Dp 's and γ^+Y are virtually always nonrecombinant chromosomes and as such almost always enter the distributive pool. The $\overline{XX}$ and $\overline{44}$ as compound chromosomes are invariable pool members. Although crossing over may occur between the two homologous arms of the $\overline{XX}$, it is necessary that independent centromeres be present on each chromosome undergoing exchange to prevent the recombinants from entering the pool (E. H. GRELL 1963).

Each female was mated to males of the genotype γ^2/Y ; $ci^D/\overline{44}$, $ci ey^R$. These males are triplo-4, since they carry a free 4 marked with a ci^D and a compound 4 marked homozygously with $ci ey^R$. The classes of progeny from these crosses are shown in Table 2. Two types of progeny are expected to predominate as a result of this cross, since in each case the distributive pool consists of only two chromosomes, which should pair and segregate regularly from each other. One type should carry the $\overline{44}$ but not the other member of the pool and be γ^2 ; ci^D or γ^2 ; $+$ in phenotype. The progeny bearing the phenotype γ^2 ; ci^D are triplo-4, carrying the free 4 of the father and the heterozygously marked $\overline{44}$ from the mother. The progeny bearing the γ^2 ; $+$ phenotype are tetra-4, carrying the heterozygously marked $\overline{44}$ from the mother and the homozygously marked $\overline{44}$ from the father. The other predominant type of progeny should carry the Dp , γ^+Y or $\overline{XX}$ but not the $\overline{44}$ and should be γ^+ ; $ci ey^R$ in the case of the Dp and γ^+Y , and $\gamma^2 sc w^a v$; $ci ey^R$ matroclinous females with the $\overline{XX}$. These progeny are diplo-4, carrying only the homozygously marked $\overline{44}$ from the father. The values obtained for this type must be doubled to compensate for the lethality of its reciprocal, which is haplo-4, $ci^D/0$ and therefore inviable. Some via-

TABLE 2

Progeny from crosses in the noncompetitive situation: Type 1 and 2 ♀♀: $y^2/y^2/Dp, y^+$ (or y^+Y); $\overline{44}♀ \times y^2/Y; ci^D/\overline{44}, ci\ ey^R ♂$. Type 3 ♀♀: $\overline{XX}, y^2\ sc\ w^a\ v$; $\overline{44}♀ \times y^2/Y; ci^D/\overline{44}, ci\ ey^R ♂$

Maternal segregation pattern	4's from ♂	4-ploidy of progeny	Phenotype of progeny
TYPE 1 (or 2) ♀			
Dp (or y^+Y)	ci^D	haplo-4	(lethal)
↕	$\overline{44}, ci\ ey^R$	diplo-4	$y^+; ci\ ey^R$
$\overline{44}$	ci^D	triplo-4	$y^2; ci^D$
	$\overline{44}, ci\ ey^R$	tetra-4	$y^2; +$
OR			
0	ci^D	haplo-4	(lethal)
↕	$\overline{44}, ci\ ey^R$	diplo-4	$y^2; ci\ ey^R$
Dp (or y^+Y); $\overline{44}$	ci^D	triplo-4	$y^+; ci^D$
	$\overline{44}, ci\ ey^R$	tetra-4	$y^+; +$
TYPE 3 ♀ *			
$\overline{XX}$	ci^D	haplo-4	(lethal)
↕	$\overline{44}, ci\ ey^R$	diplo-4	$y^2\ sc\ w^a\ v; ci\ ey^R ♀$
$\overline{44}$	ci^D	triplo-4	$y^2; ci^D ♂$
	$\overline{44}, ci\ ey^R$	tetra-4	$y^2; + ♂$
OR			
0	ci^D	haplo-4	(lethal)
↕	$\overline{44}, ci\ ey^R$	diplo-4	$y^2; ci\ ey^R ♂$
$\overline{XX}; \overline{44}$	ci^D	triplo-4	$y^2\ sc\ w^a\ v; ci^D ♀$
	$\overline{44}, ci\ ey^R$	tetra-4	$y^2\ sc\ w^a\ v; + ♀$

* Type 3 females produce only matroclinous daughters and patroclinous sons.

bility differences exist between diplo-4, triplo-4, and tetra-4 flies. Tetra-4 flies were found to possess 80% of the viability of the diplo-4 class and 68% of the viability of the triplo-4 class in these experiments. Since total segregation patterns and not their component parts are compared, and since within each segregation the three types are produced with equal frequency (Table 2), no additional viability correction for fourth chromosome aneuploidy need be applied.

When the members of the pool fail to pair, they move randomly with respect to each other. Half of the time they move to opposite poles, and half of the time they move to the same pole. In the former case the products are exactly the same as those resulting from the pairing and segregation of the pool members. In the latter case two different classes result, one in which both members of the pool are present and one in which neither is present.

When both are present, the phenotype is $y^+; ci^D$ or $y^+; +$ for the Dp and y^+Y , and $y^2\ sc\ w^a\ v; ci^D$ or $y^2\ sc\ w^a\ v; +$ macroclinous females for the $\overline{XX}$. The $y^2; ci^D$ and $y^2\ sc\ w^a\ v; ci^D$ progeny are triplo-4 and the $y^+; +$ and $y^2\ sc\ w^a\ v; +$ progeny are tetra-4. When neither is present, the phenotype is $y^2; ci\ ey^R$ from the Dp and y^+Y mothers and $y^2; ci\ ey^R$ patroclinous males from the $\overline{XX}$ mothers. These progeny are diplo-4, and again the values must be doubled to compensate for the inviable haplo-4 reciprocal.

Since half the products of random assortment result in nondisjunction of the chromosomes involved, association (a) may be calculated as $a = 1 - 2n$ where n = nondisjunction (GRELL and GRELL 1960). Nondisjunction frequencies then are inversely related to pairing; i.e., the lowest nondisjunction frequency indicates the highest amount of pairing.

Competitive situation: The competitive situation was set up to insure that three chromosomes would comprise the distributive pool. This was done by introducing each *Dp* into females with a $\overline{XX}$ (marked with $\gamma^2 sc u^a v$) and with the $\overline{44}$. These females were mated to males of the genotype $\gamma^2/Y; ci^D/\overline{44}, ci ey^R$. From this cross only matroclinous females and patroclinous males should result. In the female the $\overline{XX}$, *Dp*, and $\overline{44}$ all enter the distributive pool, and the pattern of disjunction of the three is determined in the progeny by the appropriate markers. The $\overline{XX}$ is identified by $sc u^a v$ (and is found only in matroclinous females), the *Dp* by γ^+ , and the $\overline{44}$ by ci^D and $ci ey^R$ and $+$ as indicated in the noncompetitive situation. The nondisjunction frequencies are measured for the four possible segregation patterns of the three chromosomes in the pool. As in the noncompetitive situation, the $ci ey^R$ classes must be doubled to compensate for the inviability of $ci^D/0$ classes; and similarly, differences in viability between diplo-4, triplo-4, tetra-4 progeny require no correction, since all types are produced with equal frequency in each segregation. Tetra-4 flies were found to possess 73% of the viability of the diplo-4 class and 57% of the viability of the triplo-4 class in these experiments.

In both situations, newly eclosed females were mated singly to three males for 24 hr in vials and the parents were then transferred to bottles for six days, after which they were removed. Temperature was maintained at $25 \pm 0.5^\circ\text{C}$. The culture medium was a mixture of corn meal, sugar, dried brewer's yeast, and agar.

RESULTS

Noncompetitive situation: The phenotypes of the progeny from the crosses are given in Table 3. The nondisjunction frequencies for each of the *Dp*'s, the γ^+Y , and the $\overline{XX}$ with the $\overline{44}$ are given in Table 4. The highest nondisjunction frequencies—5.47, 2.64, 2.59, and 1.57%—were obtained with *Dp 1187*, *Dp 1205*, $\overline{XX}$, and γ^+Y , respectively, which were the smallest and largest elements used. These give association values of about 89, 95, 95, and 97%, respectively. The lowest nondisjunction value, indicating the highest amount of association, was 0.16% and was obtained with *Dp 1346*, which has a length of 2.0 and is therefore virtually the same size as the $\overline{44}$. This gives an association value of about 99.7%. Thus, the nondisjunction frequencies are lowest when the *Dp*'s are most similar in size to the $\overline{44}$; so the *Dp*'s with a size ratio to the $\overline{44}$ (or the $\overline{44}$ to the *Dp*, the smaller chromosome acting as the numerator) of 0.5 or more all show nondisjunction frequencies less than 0.4%. Conspicuous increases in nondisjunction occur only when the size ratio of the $\overline{44}$ and the other element becomes smaller than 0.5, e.g. *Dp 1205*, *Dp 1187*, $\overline{XX}$, and γ^+Y (Table 4 and Figure 1). It is apparent, then, that when only two chromosomes are in the distributive pool, pairing efficiency, as based on nondisjunction frequency, is closely related to the similarity in length of the two chromosomes.

Competitive situation: In the competitive situation three chromosomes are in the distributive pool, allowing more kinds of association and segregation patterns. Instead of one type of bivalent, there are now three possible types of bivalents ($\overline{XX}$ -*Dp*; $\overline{XX}$ - $\overline{44}$; *Dp*- $\overline{44}$) and one trivalent ($\overline{XX}$ -*Dp*- $\overline{44}$). The phenotypes of the progeny from the crosses are given in Table 5. The frequencies of the different classes of progeny with respect to the segregation of the three chromosomes in the distributive pool are given in Table 6. The nondisjunction frequencies calculated from these data for the four possible segregation patterns are given in

TABLE 3
*Phenotypes of progeny from the crosses: $y^2/y^2/Dp,y^+$ (or y^+Y); $44q \times y^2/Y$;
 $ci^D/44$, $ci\ ey^R \delta$ and $\overline{XX}$, $y^2\ sc\ w^a\ v$; $44q \times y^2/Y$; $ci^D/44$, $ci\ ey^R \delta$*

DpNo	Females					Males					Totals		
	y^2		y^+		+	y^2		+		+			
	$ci\ ey^R$	ci^D	$ci\ ey^R$	ci^D		$ci\ ey^R$	ci^D	$ci\ ey^R$	ci^D				
1187	51	1048	576	966	94	38	992	565	921	69	24	5382	
1205	27	1008	585	955	22	35	938	473	917	30	14	5018	
1144	4	1008	454	949	1	4	0	961	509	895	2	1	4788
1337	1	1093	755	1053	2	11	0	1076	749	794	1	2	5537
1346	4	1902	1469	1761	4	0	2	1909	1385	1584	3	3	10026
1488	9	1048	918	1068	2	1	0	1085	802	935	0	5	5873
856	3	1142	720	1022	1	13	1	1084	647	934	3	2	5572
1173	7	1590	1169	1272	2	8	1	1491	1143	1109	3	8	7803
3	7	1268	864	1145	2	10	1	1237	759	988	3	2	6286
y^+Y	14	998	657	592	4	2	10	1014	740	591	19	19	4660
$\overline{XX}$	1369	26	13							58	1677	1425	4568

TABLE 4

Nondisjunction frequencies for the two members of the distributive pool in the noncompetitive situation

Chromosome in pool with $\overline{44}$	Length	Totals*	Size ratio of Dp and $\overline{44}$ (numerator smaller chromosome)	Nondisjunction of Dp and $\overline{44}$ (percent $\pm$ S.E.)	Association† of Dp and $\overline{44}$ (percent)
Dp 1187	≤ 0.3	7,358	0.15	$5.47 \pm .27$	89.06
Dp 1205	0.7	6,931	0.35	$2.64 \pm .19$	94.72
Dp 1144	1.1	6,636	0.55	$0.24 \pm .06$	99.52
Dp 1337	1.4	7,385	0.70	$0.24 \pm .06$	99.52
Dp 1346	2.0	13,377	1.0	$0.16 \pm .01$	99.68
Dp 1488	2.5	7,885	0.8	$0.32 \pm .06$	99.36
Dp 856	3.0	7,532	0.67	$0.36 \pm .06$	99.28
Dp 1173	3.6	10,192	0.56	$0.36 \pm .01$	99.28
Dp 3	>4	8,427	~ 0.5	$0.39 \pm .07$	99.22
$\gamma+Y$	~ 9	5,867	~ 0.2	$1.57 \pm .17$	96.86
$\overline{XX}$	~ 20	5,995	~ 0.1	$2.59 \pm .21$	94.82

* Totals inflated by correction for inviability of $c\overline{i}^D/0$ progeny.

† Values calculated from segregation data using the formula $a = 1 - 2n$, where a = association and n = nondisjunction.

FIGURE 1.—Comparison of Dp , $\overline{44}$ nondisjunction in competitive and noncompetitive situations.

TABLE 5

*Phenotypes of progeny from the crosses: $\overline{XX}, y^2 sc w^a v/Dp(1:f), y^+$;
 $44 \varphi \times y^2/Y; ci^D/44, ci ey^R \delta$*

Dp Number	Females					Males					Totals	
	$y^2\ sc\ w^a\ v$		$y^+\ sc\ w^a\ v$		+	y^2		y^+		+		
	$ci\ ey^R$	ci^D	$ci\ ey^R$	ci^D		$ci\ ey^R$	ci^D	$ci\ ey^R$	ci^D			
1187	144	131	82	6	1	105	184	110	11	900	621	3039
1205	104	191	101	0	34	149	157	109	2	1017	621	3243
1144	5	340	150	0	2	330	11	12	0	991	591	3342
1337	4	279	144	0	1	178	3	6	1	953	622	3069
1346	0	468	174	4	0	286	4	3	6	1065	574	3424
1488	3	599	268	11	0	424	21	5	12	731	397	3001
856	4	594	278	10	1	437	24	7	11	629	409	2886
1173	12	784	318	0	6	430	16	14	1	646	347	3080
3	43	879	500	0	0	780	44	16	2	730	525	4099

TABLE 6

Progeny from the cross $\overline{XX}, y^2 sc w^a v/Dp(1:f), y^+; 44 \varphi \times y^2/Y; ci^D/44, ci ey^R \delta$ in the competitive situation

Chromosome	Length	Totals	Classes of progeny with respect to segregation of $\overline{XX}, Dp$ and 44						$\overline{XX}, Dp, 44 \leftrightarrow$					
			$\overline{XX}, Dp^*$		$\leftrightarrow$		Dp^*		$Dp, 44$		$\leftrightarrow$		$\overline{XX}, Dp, 44$	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Dp 1187</i>	≤ 0.3	4,043	1488	36.80	1521	37.62	213	5.27	210	5.19	294	7.28	288	7.12
<i>Dp 1205</i>	0.7	4,256	1516	35.62	1638	38.49	292	6.86	298	7.00	266	6.25	208	4.89
<i>Dp 1144</i>	1.1	4,587	1820	39.68	1582	34.49	490	10.68	660	14.39	23	0.50	10	0.22
<i>Dp 1337</i>	1.4	4,130	1756	42.51	1575	38.14	423	10.23	356	8.62	9	0.22	8	0.20
<i>Dp 1346</i>	2.0	4,556	1680	36.87	1639	35.97	642	14.09	572	12.55	7	0.16	0	0
<i>Dp 1488</i>	2.5	3,970	1060	26.70	1128	28.41	867	21.84	848	21.36	26	0.65	6	0.15
<i>Dp 856</i>	3.0	3,820	964	25.24	1038	27.17	872	22.83	874	22.88	31	0.81	8	0.20
<i>Dp 1173</i>	3.6	4,029	1012	25.12	993	24.65	1102	27.35	860	21.35	30	0.74	24	0.60
<i>Dp 3</i>	> 4	5,504	1160	21.06	1255	22.80	1379	25.05	1560	28.34	60	1.10	86	1.57
													0	0

* Counts inflated by correction for inviability of $ci^D/0$ progeny.

TABLE 7

Nondisjunction frequencies in competitive situation

Chromosome	Length	Totals*	Nondisjunction for three competitors (percent)			
			<i>Dp</i> (or <i>Y</i>), $\overline{44}$	$\overline{XX}$, $\overline{44}$	$\overline{XX}$, <i>Dp</i> (or <i>Y</i>)	$\overline{XX}$, <i>Dp</i> (or <i>Y</i>), $\overline{44}$
<i>Dp</i> 1187	≤ 0.3	4,043	14.40	10.46	74.42	0.72
<i>Dp</i> 1205	0.7	4,256	11.14	13.86	74.11	0.89
<i>Dp</i> 1144	1.1	4,587	0.72	25.07	74.17	0.04
<i>Dp</i> 1337	1.4	4,130	0.42	18.86	80.65	0.07
<i>Dp</i> 1346	2.0	4,556	0.16	26.64	72.84	0.36
<i>Dp</i> 1488	2.5	3,970	0.80	43.20	55.11	0.89
<i>Dp</i> 856	3.0	3,820	1.01	45.71	52.41	0.87
<i>Dp</i> 1173	3.6	4,029	1.34	48.70	49.77	0.19
<i>Dp</i> 3	>4	5,504	2.67	53.39	43.86	0.08
<i>B^sY</i>	~ 8	1,406	26.5	63.1	10.2	0.2

* Totals inflated by correction for inviability of *ci^D/0* progeny.

Table 7. The values for the *B^sY* are from E. H. GRELL (unpublished). As in the noncompetitive situation, the lowest nondisjunction frequency for a *Dp* and the $\overline{44}$ is with *Dp* 1346 (0.16%, Table 7, Column 4) with values increasing as the *Dp*'s become smaller or larger than 2.0. The values increase more rapidly in the competitive situation than in the noncompetitive one. The smallest and largest *Dp*'s (and *B^sY*) show a marked increase in the nondisjunction values over those obtained in the noncompetitive situation, as shown in Figure 1, which compares the *Dp* (or *Y*) and $\overline{44}$ nondisjunction frequencies in the competitive and noncompetitive situations. For *Dp*'s of size 1.1 to > 4 (i.e., when the sizes of the two smaller chromosomes are in a ratio of 0.5 or greater), the $\overline{XX}$ in the competitive situation has a limited effect on the *Dp*, $\overline{44}$ nondisjunction; the values in this region are quite similar in both the competitive and noncompetitive situations. But when the ratio of the *Dp* (or the *B^sY*) and $\overline{44}$ is less than 0.5, the $\overline{XX}$ is a much more effective competitor, greatly increasing the nondisjunction frequency of the two smaller chromosomes.

Figure 2 shows the relationship of the nondisjunction frequency to *Dp* length for the three pairwise combinations of the *Dp*, $\overline{44}$, and $\overline{XX}$ in the distributive pool. The *Dp*, $\overline{44}$ nondisjunction frequencies have been discussed in the preceding paragraph. The $\overline{XX}$, *Dp* nondisjunction values for the small *Dp*'s (sizes 0.3–2.0) are over 70%. After the *Dp* size of 2.0 is reached, this type of nondisjunction decreases as the *Dp* sizes increase, reaching the lowest value of 10.2% with the *B^sY* chromosome. The $\overline{XX}$, $\overline{44}$ nondisjunction frequencies are almost the reciprocal of this. For the small *Dp*'s (sizes 0.3–0.7), a plateau of less than 15% is formed. With the *Dp* of size 1.1 a rise begins and increases to the highest value, 63.1%, with the *B^sY* chromosome. *Dp* 1337 (size 1.4) shows a deviation from the pattern established by the other *Dp*'s for both the $\overline{XX}$, *Dp* and $\overline{XX}$, $\overline{44}$ nondis-

FIGURE 2.—Relation of nondisjunction frequencies to duplication length in the competitive situation.

junction frequencies. This could be the result of viability differences among the different classes of progeny when *Dp* 1337 is present, as suggested by other situations involving this *Dp* (GRELL 1964a).

Trivalents with a directed assortment of chromosomes are necessary to explain nondisjunction frequencies greater than 50%, since strictly bivalent-univalent formations can give frequencies no higher than 50%. Genetic data alone only provide an estimate of the minimal number of trivalents required to explain the data. The possibility cannot be excluded that trivalents invariably occur, in which case size-determined disjunction rather than size-determined association would account for the segregation results. This premise appears incompatible with earlier studies and with the present data which show that invariable bivalent association does not occur in the noncompetitive situation. Since there is compelling evidence to show that with two members bivalent association is size dependent, it is most reasonable to assume that in the competitive situation, bivalent association between the two most unlike members is negligible. This assumption leads to a minimal estimate for the frequency of trivalents and a maximal estimate for the frequency of bivalents (see GRELL 1964a Table 4). Using such minimal estimates, trivalents are necessary to explain $\overline{XX}$, *Dp* nondisjunction frequencies for *Dp*'s of size ≤ 0.3 to 3.0 and $\overline{XX}$, as well as $\overline{44}$, nondisjunction frequencies for the *Dp* of size > 4 and for the $B^S Y$. It has been shown that the directed assortment from a trivalent composed of three ele-

ments of different sizes is such that the chromosome of intermediate length tends to direct the other two chromosomes to the same pole, while it moves alone to the opposite pole (GRELL 1964a). Thus it must be assumed that for Dp 's smaller than 3.6 in size the $\overline{44}$ acts as the intermediate element, directing the $\overline{XX}$ and Dp to the same pole, causing the $\overline{XX}$, Dp nondisjunction values to exceed 50% and the $\overline{XX}$, $\overline{44}$ values to remain smaller. For Dp 's greater than 3.6 the Dp acts as the intermediate element, directing the $\overline{XX}$ and $\overline{44}$ to the same pole. Therefore, the $\overline{XX}$, Dp nondisjunction frequencies fall below 50% while the $\overline{XX}$, $\overline{44}$ frequencies exceed this value.

Both $\overline{XX}$, Dp and $\overline{XX}$, $\overline{44}$ nondisjunction approach 50% when the Dp size reaches 3.6. This indicates that the Dp and $\overline{44}$ are segregating regularly, while the $\overline{XX}$ is moving randomly with respect to both. This, however, is not the point at which maximum Dp , $\overline{44}$ segregation occurs; it occurs with the Dp of size 2.0 in both the competitive and noncompetitive situations, perhaps suggesting a difference in the criteria for pairing and for disjunction.

Maximum trivalent formation, as indicated by maximum nondisjunction frequencies is postulated when the size of the intermediate chromosome is the geometric mean of the other two, i.e. when $b = \sqrt{ac}$ (GRELL 1964a). Thus, assuming a length of ~ 20 for the $\overline{XX}$, two maxima might be expected—one when the Dp is the geometric mean of the $\overline{XX}$ and $\overline{44}$ (at ~ 6.3) and one when the $\overline{44}$ is the geometric mean of the $\overline{XX}$ and Dp (at ~ 0.2). The length given the $\overline{XX}$ is only approximate, and therefore the values calculated for maximum trivalent formation are likewise only approximate, since these values vary with the length given the $\overline{XX}$. The maximum at 0.2 is less than the length for any Dp used in this study and therefore cannot be considered with any certainty. The calculated maximum at 6.3 is near the observed maximum obtained with the B^sY . If a Dp of the proper size (6.3) had been studied, the value obtained for it might equal or exceed that for the B^sY and thus represent the maximum. This point, at which maximum trivalent formation is assumed to occur, is close to the point where the curves representing $\overline{XX}$, Dp and Dp , $\overline{44}$ nondisjunction cross (6.7). This relationship suggests that when the length of the intermediate chromosome, in this case the Dp , is the geometric mean of the other two chromosomes, it associates with both equally well, and the greatest amount of trivalent formation occurs. Accordingly, the maximum at 0.2 should coincide with the point where the curves representing $\overline{XX}$, $\overline{44}$ and Dp , $\overline{44}$ nondisjunction cross. The observed point (0.5) is close but not coincident, possibly because of the approximate value given to the $\overline{XX}$. If the size of the $\overline{XX}$ were precisely known, the calculated maximum might coincide more closely with the points at which the curves cross.

The comparison of Dp , 4 nondisjunction with Dp , $\overline{44}$ nondisjunction for the noncompetitive situation is shown in Figure 3 and for the competitive situation in Figure 4. The data for the Dp , 4 nondisjunction in both situations was taken from R. F. GRELL (1964a). If recognition is dependent on arm length in the dis-

FIGURE 3.—Comparison of $Dp, 4$ and $Dp, \overline{44}$ nondisjunction in the noncompetitive situation. ($Dp, 4$ data from R. F. GRELL 1964a)

tributive pool, the two sets of data should coincide, since arm lengths for the 4 and the $\overline{44}$ are about the same; but if total length is the recognition factor the two sets of data should be displaced, since the total length for the $\overline{44}$ is about twice that for the 4.

In the noncompetitive situation (Figure 3), the lowest $Dp, 4$ nondisjunction value, 0.02%, is obtained with the Dp of size 1.1, and the values increase with Dp 's whose sizes are larger or smaller than this, indicating that pairing and segregation are greatest when the Dp is closest to the 4. The $Dp, \overline{44}$ nondisjunction values follow this same pattern, but the lowest value, 0.16%, is obtained with the Dp of size 2.0. The $Dp, \overline{44}$ curve, which is very similar to the one for $Dp, 4$ nondisjunction, is displaced to the right by about one unit, which represents the difference in size between the 4 and $\overline{44}$. Thus pairing and segregation are greatest for the $\overline{44}$ when the Dp is size 2.0 and greatest for the 4 when the Dp is size 1.1, indicating that the size for recognition and segregation is determined by total length.

The same pattern is apparent when $Dp, 4$ and $Dp, \overline{44}$ nondisjunctions are compared in the competitive situation (Figure 4). Here again, the two curves are

FIGURE 4.—Comparison of $Dp, 4$ and $Dp, \overline{44}$ nondisjunction in the competitive situation. ($Dp, 4$ data from R. F. GRELL 1964a).

similar but $Dp, \overline{44}$ is again displaced by about one unit. The lowest nondisjunction value for the Dp 's with the 4 is 0.07%, obtained with the Dp of size 1.0. For the Dp 's with the $\overline{44}$, the lowest nondisjunction value is 0.16%, obtained with the Dp of size 2.0. Therefore in the presence of a competitor the size for both recognition and segregation of these two elements is again determined by total length

DISCUSSION

The present experiments have shown that, with a metacentric, total length rather than arm length is the recognition factor in pairing. When the nondisjunction frequencies obtained for the Dp 's with a 4 and with a $\overline{44}$ in the noncompetitive and competitive situations (Figures 3 and 4) are compared, the curves are virtually identical but are displaced by about one unit. The lowest $Dp, \overline{44}$ nondisjunction frequencies in the noncompetitive and competitive situations are identical and are obtained with the Dp closest in size to the $\overline{44}$ (2.0), in

contrast to previous results where the lowest Dp , 4 nondisjunction values in both situations were obtained with the Dp closest in size to the 4 (~ 1.0). If arm length had been the criterion, the two sets of nondisjunction frequencies would have been superimposed and the lowest Dp , $\overline{44}$ nondisjunction values obtained with the Dp of about size 1.0. The displacement of the curves by one unit, representing precisely the difference in total length between the 4 and $\overline{44}$, indicates that the $\overline{44}$ is recognized as an element twice as long as the 4 rather than as an element of equal length.

Previous experiments involving a noncompetitive and a competitive situation indicated that pairing efficiency and preference are dependent on the size similarity of the chromosomes involved (GRELL 1964a). In the noncompetitive situation, when only two chromosomes—a free 4 and $X-Dp$ —were in the pool, the frequency with which the two paired was a function of their similarity in size. Thus the Dp of size 1.1 produced the greatest amount of pairing with the 4, and the frequency of pairing decreased as the Dp 's became larger or smaller than this. The same pattern is seen in the present noncompetitive situation involving a $\overline{44}$ and Dp . Here, however, the greatest amount of pairing is with the Dp of size 2.0. The logarithmic scale on which the chromosome lengths were plotted indicates, by the symmetry of the curves, that the ratio of lengths rather than absolute lengths determines pairing efficiency.

Likewise, the earlier experiments showed that for the competitive situation the ratio of sizes of the three chromosomes determined the pairing efficiency and preferences of the chromosomes involved. The addition of a third element, the T_4 , to the pool as a competitor with the 4 and Dp had little effect when the sizes of the two smaller elements were in a ratio of 0.67 or greater. Only when the ratio of the 4 to Dp was less than 0.67 was the T_4 effective as a competitor. In the present experiments the competitor $\overline{XX}$ is effective only when the $\overline{44}$ and Dp 's have size ratios of less than 0.5. The difference in ratios undoubtedly reflects the difference in the relative sizes of the competitors and the two smaller chromosomes with which they are competing. The T_4 is not quite six times the length of the 4, while the $\overline{XX}$ is about ten times the length of the $\overline{44}$. The larger size of the $\overline{XX}$ in relation to the two smaller chromosomes makes it less effective as a competitor than the T_4 . Hence the T_4 is effective when the two smaller chromosomes are in ratio of 0.67 or less, while the $\overline{XX}$ does not become effective until a ratio of 0.5 or less is reached. Within the ranges of 1 to 0.67 or 1 to 0.5 the two smaller chromosomes tend to pair and segregate regularly, while the larger chromosome has little effect on their disjunction. Outside these ranges the larger chromosome becomes a more effective competitor, and the nondisjunction between the two smaller chromosomes increases as the difference between their lengths increases.

Trivalent formation also depends on the size ratio of the chromosomes. The earlier experiments (GRELL 1964a) showed that during disjunction from trivalents, the chromosome of intermediate size tended to direct the other two chro-

mosomes to the same pole. With Dp 's less than 1.3 in size the 4 acted as the intermediate element, directing the T_4 and Dp to one pole; with Dp 's greater than 1.3 in size the Dp became the intermediate, directing the 4 and T_4 to the same pole. Intermediacy, as judged by disjunctive behavior, coincided well with the physical transition to intermediate size. In the present studies, however, coincidence between behavioral and physical transition points is poor. The switch point, if it were to coincide with the physical change in size, should occur at 2.0; but it actually occurs at 3.6. A comparison of the present situation with the earlier one reveals one basic difference between the chromosomes concerned. In the present study one of the two smaller chromosomes is a metacentric, while in the previous study both were acrocentrics. Conceivably, the possession of two arms enhances the ability of a chromosome to serve as an intermediate in disjunction, superceding to some degree its lack of size qualification for the role. Limitations do exist, since the ability extends only to the point where the size ratio of the smaller metacentric to the larger acrocentric is 2:3.6. Beyond this point the acrocentric assumes the role that accords with its physical size. (For further data and discussion of this subject, see the second paper in this series.)

These observations permit a distinction to be drawn between the recognition and disjunctive properties of chromosomes in the distributive process. Pairing frequency between the Dp and the $\overline{44}$ conforms precisely to size similarity in both the noncompetitive and competitive situations, being greatest for the Dp closest in size to the $\overline{44}$. By contrast, the metacentric $\overline{44}$ continues to act as the intermediate in disjunction, despite its smaller size, up to the point where the acrocentric Dp is nearly twice the total length of the $\overline{44}$.

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