LOCALIZATION OF THE GENETIC FACTORS RESPONSIBLE FOR THE KINETIC ACTIVITY OF X CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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THE genetic analysis of kinetic activity in *Drosophila melanogaster* based on the fate of first anaphase bridges derived from double crossing over within heterozygous inversions made it possible for centromeres to be characterized as weak or strong. The experimental basis for the distinction between these two types was described earlier (NOVITSKI 1952) and a more detailed description of the genetic consequences of anaphase bridge formation at both first and second meiotic divisions is presented elsewhere (NOVITSKI 1955).

Centromeres derived from a normal X chromosome are weak; whereas those found on detachments of attached–X chromosomes are strong when they carry the long arm of the Y chromosome. Some centromeres carrying the short arm of the Y chromosome are weak and some are strong. In general, the method of replacing one centromere with another involves heterochromatic exchange, with a consequent alteration of the associated heterochromatin. Of considerable genetic interest would be a determination of whether the genetic results that have been attributed to "centromere activity" depend on the nature of the centromere itself, or on the constitution of the adjacent heterochromatic regions from which the centromere is ordinarily inseparable. The experiments to be described here, designed to distinguish between these two possibilities, indicate that the latter is true.

Detachments from duplication bearing females

In the first set of experiments to be discussed, the free Y chromosome ordinarily present in an attached–X female was replaced by a small duplication that includes the euchromatic tip and proximal heterochromatin of the X. The attached-X was of special construction, carrying the fertility factors of the long arm of the Y chromosome interstitially (Figure 1). Females were irradiated and detachments carrying the long arm of the Y, which were recovered in their progeny, were analyzed genetically to determine their composition; in some instances the detachment originated by exchange with the fourth chromosome
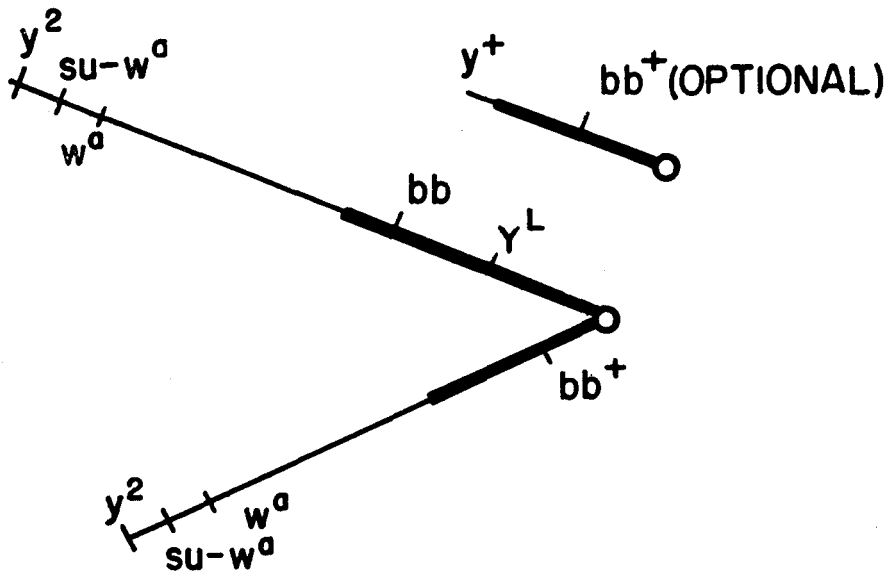
FIGURE 1.—Specially constructed attached-X carrying Y^L in one arm, and a free X duplication from which detachments used in tests were derived.

rather than with the duplication. Those detachments whose genetic composition indicated that they might prove valuable for further analysis were then tested in the usual way against both a standard weak centromere and a standard strong centromere.

When the centromeres at opposite ends of a bridge are both weak, the expected ratio of recovered male crossover progeny to patroclinous males is about 3:2; when both are strong the ratio is about 3:0; and when there is one of each type, the ratio is about 3:1. Observed ratios generally deviate from the expected owing to a slight excess of patroclinous males arising in part at least from primary nondisjunction. In principle any unknown can be characterized by a single combination with either a known weak, or a known strong. In all cases presented here, however, unknowns were tested against both a known weak and a known strong for more reliable determinations of strength. Cytological examination was made in the secondary spermatocyte, where a terminal centromere can be most readily distinguished from a subterminal one.

The method of presentation of the data in Table 1 is that used previously (NOVITSKI 1952). The structural formulas of the heterozygous females tested give the sequence of the components of the new chromosome type above the line, and the standard, with or without Y^L, below the line. The raised dot indicates the relative position of the centromere among the components of the detachment. The order of components included within parentheses is ambiguous. The cases listed will be discussed individually. Excluded from this discussion is an equal number of tests that contributed nothing essentially new to the problem.

Detachment 164–9: This detachment behaves in a typically weak fashion,

TABLE 1

Genetic results from inversion heterozygotes

Test	Maternal composition		F1, Noncrossover and double crossover regions							Totals		Ratio
	Structural	Genetic	0	1,2	1,3	1,4	2,3	2,4	3,4	co	Pat	as 3:x
164–9	XYᴸ· / sc⁸	y² wᵃ	417	0	1	2	9	7	5	43	31	3:2.1
		f v cv	300	0	1	3	6	6	3			
	XYᴸ· / sc⁸·Yᴸ	y² wᵃ	577	3	14	5	27	18	13	162	62	3:1.1
		car f v cv	574	4	23	10	17	24	4			
122–19	XYᴸ·4 / sc⁸	y² wᵃ	1940	4	17	4	25	39	21	175	115	3:2.0
		f v cv	1184	1	17	7	16	18	6			
	XYᴸ·4 / sc⁸·Yᴸ	y² wᵃ	728	1	8	6	21	17	3	132	43	3:1.0
		car f v cv	713	10	15	8	20	17	6			
179–8	X(·Yᴸ)4 / sc⁸	y² wᵃ	859	2	5	1	22	20	9	99	40	3:1.2
		f v cv	489	0	11	5	10	15	3			
	X(·Yᴸ)4 / sc⁸·Yᴸ	y² wᵃ	1035	6	13	10	27	18	10	168	11	3:0.2
		car f v cv	650	9	28	5	27	12	4			
3–18	X(Yᴸ·) / sc⁸	y² wᵃ	2402	5	19	16	58	60	26	298	101	3:1.0
		f v cv	1510	5	20	20	24	36	9			
	X(Yᴸ·) / sc⁸·Yᴸ	y² wᵃ	1317	8	26	7	45	23	10	240	14	3:0.2
		car f v cv	1212	12	35	7	33	19	4			
118–12b	X(Yᴸ·) / sc⁸	y² wᵃ	2438	5	15	10	39	27	13	202	71	3:1.1
		f v cv	1542	8	22	11	28	18	6			
	X(Yᴸ·) / sc⁸·Yᴸ	y² wᵃ	1037	1	11	15	22	6	1	92	9	3:0.3
		car f v cv	891	6	14	13	18	4	0			
174–13	XYᴸ· / sc⁸	y² wᵃ	1015	0	6	5	19	27	15	128	69	3:1.6
		f v cv	716	3	5	8	14	20	6			
	XYᴸ· / sc⁸·Yᴸ	y² wᵃ	507	2	16	9	19	26	5	154	37	3:0.7
		car f v cv	404	3	22	15	14	18	5			
9–10r	X·4 / N*	B y	912	6	32	21	25	29	3	239	52	3:1.3
		sc cv v f	1024	10	26	22	37	24	4			

Cross	Chromosome	Progeny										Ratio
9–10e	X4. By	141	0	20	8	21	24	5		139	10	3:0.4
	N.YL sc cv v f	125	3	14	8	11	17	8				
	X4. By	3322	24	94	42	106	50	22		638	182	3:1.7
	N sc cv v f	2504	22	90	43	72	55	18				
9–10o	X4. By	967	4	36	14	26	16	5		199	23	3:0.7
	N.YL sc cv v f	923	7	28	12	28	13	10				
	X4. By	1190	7	24	6	29	23	5		189	51	3:1.6
	N sc cv v f	1054	6	34	13	19	22	1				
	X4. By	1158	6	24	8	18	18	2		165	15	3:0.5
	N.YL sc cv v f	989	2	29	13	27	14	4				
9–24d	X4. By	816	5	25	5	20	17	8		171	47	3:1.8
	N sc cv v f	840	6	32	9	27	12	5				
	X4. By	929	1	21	15	30	16	4		164	33	3:1.3
	N.YL sc cv v f	817	4	15	5	28	22	3				
9–20i	X4. By	3016	19	63	50	96	97	40		710	192	3:1.6
	N sc cv v f car	2761	15	110	54	85	55	26				
	X4. By	2062	5	47	20	62	51	26		375	103	3:1.6
	N sc cv v f	1852	11	42	14	50	36	11				
	X4. By	1517	15	73	15	71	61	24		483	70	3:1.1
	N.YL sc cv v f	1379	7	60	30	65	45	17				

* N = normal sequence.

Region 1, y-cv; region 2, cv-v; region 3, v-f; region 4, f-sfα; progeny from each cross are tabulated in two rows, with the individuals in each row carrying the chromosome of the structure designated by the corresponding row in the maternal structural composition. The paternal genotype in tests 164–9 through 174–13 was $In(1)$dl-49, y w B; consequently, only male progeny were scored. The paternal genotype in tests 9–11b through 9–20i was y w^a cv v f and all progeny were scored. To compensate for twice as many crossovers being scored in the latter tests, the patroclinous male class has been doubled in obtaining the 3:x ratio.

giving a 3:2 (3:2.1) ratio with sc^8 and a 3:1 (3:1.1) ratio with $sc^8.Y^L$. Cytologically, it has a terminal centromere, presumably an X centromere derived from the duplication, and is of interest because it represents the first case in which a detachment carrying the fertility factors of Y^L is not also strong.

Detachment 122–19: This case is typically weak in both tests. It arose by an exchange between the attached-X and the fourth chromosome, which resulted in a detachment that retains the centromere of the attached-X, but in which the tip of the fourth replaces the arm not carrying Y^L. It confirms the point made in the preceding case that the presence of Y^L does not necessarily result in a strong centromere and further shows that there is no inherent "strongness" to the centromere of this particular attached-X.

Detachment 179–8: Like 122–19, the tip of the fourth chromosome has replaced one of the arms of the attached-X, so that the centromere of the attached-X is retained in the detachment. Crossover tests show it, unlike 122–19, to be strong. Genetically, another difference between these two is that 179–8 carries the normal allele of bobbed whereas the preceding one carries the recessive allele. The possible significance of this will be discussed.

Detachments 3–18 and 118–12b: Both of these are clearly strong. They carry Y^L and cytological examination shows both to have a subterminal centromere, indicating that they still retain the centromere of the attached-X. Both carry the normal allele of bobbed.

Detachment 174–13: This is an interesting case because the ratios from both tests are abnormal, the values being intermediate between weak and strong. Ordinarily, observed ratios belonging to the 3:2 category vary from 3:2.0 to 3:2.4, those belonging to the 3:1 class vary from 3:0.9 to 3:1.4 and the 3:0 expected usually appears as a ratio between 3:0.1 and 3:0.4. As pointed out previously (NOVITSKI 1952), the distribution of these values is sharply discontinuous. The two values from 174–13, 3:1.6 and 3:0.7, are both in an atypical region of the distribution. At a later date this test was repeated on a somewhat larger scale; the results were in good agreement with those given in Table 1. The over-all ratios for the combined data were 3:1.68 and 3:0.66.

Cytological examination shows this detachment to have a terminal centromere. It seems probable that this centromere originated from chromosome four, since the duplication used to detach the attached-X chromosomes was shown later to have a centromere derived from chromosome four. It might be noted, incidentally, that intermediate values such as these may be more widespread than the present data indicate; PROFESSOR STANLEY ZIMMERING (unpublished) pointed out the occurrence of such values in experiments involving other types of chromosomes.

Two other detachments (118–2b and 122–13) were analyzed in this series; the data are not presented here because they give typically weak values and add nothing to the arguments already presented. It should be noted, however, that on genetic analysis these detachments were shown to carry the recessive allele of bobbed from the attached-X, like all the other weak ones and were unlike the strong ones, which all carried the normal allele of bobbed.

Detachments from nonduplication-bearing females

The next set of experiments to be described involves detachments obtained in a somewhat different way; an attached-X whose arms are in inverted sequence and also carry the short arm of the Y chromosome distally was used. Females with such a chromosome, but lacking a Y chromosome were irradiated, and detachments were collected. It is known that under such conditions the fourth chromosome is predominantly involved (PARKER 1954; MULLER and HERSKOWITZ 1954). The desired product was the one that would carry the centromere of the fourth chromosome, and not its tip. Consequently, matings of the detachments were made to sv^n, which genetically marks the tip of the fourth, and those detachments (numbering 25) which proved to carry its normal allele were discarded. Of 35 others, 24 showed a high production of Minute (haplo-IV) progeny, indicating that part of the fourth chromosome was involved in the detachment and was interfering with the normal disjunction of the free fourth chromosomes. Seven of these were selected for further testing, as well as one that did not produce typical haplo-IV progeny but rather did produce a weak Minute type.

Detachment 9–10r: Cytologically, this detachment has a terminal centromere. That this is the centromere of the fourth is shown not only by the production of haplo-IV progeny, but also by the fact that such haplo-IV individuals carry the detachment, indicating that one of the fourth chromosomes occasionally pairs with the detachment and disjoins from it. This insures that the class getting the homolog of the detachment will also get the fourth, whereas that class getting the detachment may or may not get the other unpaired fourth. It is clearly strong (Table 1).

Detachment 9–10e: This is the case that produces weak Minutes instead of typical haplo-IV types. It is also the one case in which we could demonstrate the normal allele of a fourth chromosome marker other than shaven; this shows that the detachment consists of the centromere and the basal section of the fourth chromosome, up to some point between the loci of ci and ey, attached to the X chromosome. The centromere is weak, although the ratios seem somewhat depressed as in 174–13.

The remainder of the tests carried out were essentially duplicates of those already described. Cases 9–10g, 9–11b, and 9–11a were kinetically similar to 9–10r, which is typically strong, but differed from it in having subterminal centromeres. Cases 9–10o, 9–20i, and 9–24d, similar to 9–10e and 174–13, appeared weak, and also gave a somewhat depressed 3:2 ratio; they have terminal centromeres.

DISCUSSION

It is now possible to present a simple, consistent picture of the contribution of the chromosome to its kinetic activity. In the first place, it is clear that centromeres as such have no specific properties as measured by the anaphase bridge tests described in this and previous works. If they did have such properties, consistent results would be expected from a given type of centromere, but in both sets of experiments described here, detachments with centromeres of similar

origin behave quite differently. In set one, both 122–19 and 179–8 must carry the centromere present on the attached-X, yet one is weak and the other strong; similarly in the second set, 9–11b and 9–10e carry the fourth centromere and behave differently.

If the centromeres of the different chromosomes of the complement had different activity, the level of this activity might be related to the physical size of the chromosome. Previous observations were consistent with this thought, since normal X centromeres were invariably weak relative to those found on detachments; whereas those that proved to be strong were derived from attached-X chromosomes and were probably ultimately derived from the Y chromosome. On this basis, the fourth might be predicted to be weaker than the X, since the small dot chromosome should not require so high a level of kinetic activity as the much larger X chromosome. It is shown, however, that the detachments with the fourth centromere may be about equivalent in strength to the X centromere (174–13, 9–10e, 9–10o, 9–20i, and 9–24d) or equivalent to the standard strong (9–10r).

It seems definite, therefore, that the differences in kinetic activity being measured in these anaphase bridge experiments cannot be referred to some property of the centromere as a point, but that its activity is being modified by its association with genetic factors. It has been shown (NOVITSKI 1952) that the kinetic property of the chromosome must reside in the vicinity of the centromere. Furthermore, when detachments are produced, this region contains indeterminate and variable amounts of heterochromatin from both the attached-X and the other chromosome participating in the detachment process. A completely reasonable conclusion is that the detachments analyzed here differ in their behavior because they contain different combinations of those elements responsible for the kinetic activity of the chromosome. The absence of suitable techniques for analyzing the heterochromatic regions of chromosomes in Drosophila makes it difficult to become more specific. Nevertheless, some of the cases do indicate that the detachment process involves a redistribution of such factors that gives rise to new situations on the detached chromosome. In the first set of experiments described here, all the detachments were tested for the presence or absence of the normal allele of bobbed. All three detachments with strong centromeres carried the normal allele of bobbed and were therefore probably duplicated for the bobbed region (see Figure 1) and conversely, the five that were not strong carried only the recessive allele. This can be considered evidence that, in this particular attached-X, a factor (or factors) imparting strength to the centromere was located so close to the locus of the normal allele of bobbed that the detachment process failed to separate them. It is conceivable that, in this test, strength is correlated with the dosage of some factor close to the locus of bobbed; however, no such correlation between the bobbed constitution and kinetic activity could be demonstrated in the second set of detachments. An explanation of this difference between the two sets of detachments may reside in the different heterochromatic constitution of the initial attached-X's.

The occurrence in these experiments of several "atypical cases" in which the ratio after test with a weak centromere is depressed somewhat below the 3:2 expectation may have some significance in this connection. 174–13, 9–10e, 9–10o, 9–20i, and 9–24d can be explained as instances in which the redistribution of the factors responsible for the kinetic activity was such that these cases represent essentially new combinations that have no counterpart in previous tests. They resemble the weak type yet consistently deviate from the 3:2 and 3:1 ratios in an unexpected direction; it should be noted that in these five cases the centromere of chromosome four has been attached to the X. It could be argued that the kinetic behavior of these centromeres is not strictly weak but is intermediate between weak and strong; thus in a certain proportion of the bridges the centromeres might behave as if they were strong. This would have the effect of shifting a 3:2 ratio in the direction of a 3:1 and a 3:1 toward a 3:0, and would fit the observations. However, the possibility is not excluded that this behavior is the result of some condition other than the one given; e.g., negative chromatid interference or an abnormally high incidence of primary nondisjunction in the females.

The data available suggest that the time of action of the system responsible for kinetic activity at the first meiotic division is subsequent to the time of genetic exchange, i.e., that the constitution of the bridge and not the constitution of the oocyte determines observed results. In an extensive series of experiments, we attempted to alter the kinetic activity of previously studied combinations by manipulating the heterochromatic constitution of the oocytes in which the bridges were formed. We modified the acentrics produced as a concomitant of bridge formation by attaching the short arm of the Y chromosome to the distal end of one or both chromosomes, or by using the terminal regions of different scute inversions. The addition of free heterochromatic duplications of X or Y origin to the heterozygous females being tested was another method used for alteration of the heterochromatic constitution of the oocyte. In no instance did the bridges behave in the way they would have if these same segments had been attached to the centromere region. The interpretation of this result is that the factors that are effective in a determination of the kinetic level are those actually associated physically with the centromere, and that those present on independent chromosomes, or on the acentric fragments produced after the exchanges that produce the bridges, are ineffective. This conclusion is similar to the one reached by RHOADES (1952) in his analysis of neocentromeres in maize. Another point of similarity in these two analyses comes from the apparent synergistic action of certain types of weak centromeres that together produce a strong effect or produce a strong effect in combination with a known strong but not with a known weak centromere (see NOVITSKI 1952). One interpretation of this effect is that there is a flow along the dicentric chromosome of some product of the centric regions from one end of the bridge to the other that enhances the activity of the weak centromeres. This resembles the movement of fiber-forming substance from the centric regions to the knobs of the chromosomes postulated by RHOADES to explain the formation of neocentromeres in maize.

SUMMARY

The genetic analysis of new X chromosome types of *Drosophila melanogaster* leads to the following conception of the genetic contribution to kinetic activity. Chromosomes do not play a passive role in their progression to the poles; they contribute autonomously to the formation of the spindle or to the process of progression, and the magnitude of this contribution differs for different chromosomes. The function of the centromere is constant from chromosome to chromosome, and it is even possible that the centromere is an inactive anchor point on the chromosome to which the spindle fiber becomes attached. The measurable differences in kinetic activity are attributable to the constitution of the heterochromatic regions of the chromosomes immediately adjacent to the centromere.

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