THE RESPONSE OF DOPA DECARBOXYLASE ACTIVITY TO VARIATIONS IN GENE DOSAGE IN DROSOPHILA: A POSSIBLE LOCATION OF THE STRUCTURAL GENE

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Manuscript received July 17, 1974

ABSTRACT

A location of the structural gene(s) for dopa decarboxylase (EC 4.1.1.26) is proposed on the basis of enzyme determinations in a set of duplication-bearing aneuploids, which revealed only one dosage-sensitive region in the *Drosophila* genome. This region lies between 36EF and 37D on the left arm of chromosome *2*.

THE activity of certain enzymes involved in the sclerotization (hardening) of the cuticle of insects changes dramatically during the ontogeny of the organism. This is best illustrated by the enzyme dopa decarboxylase, which has been studied in a number of families of the higher Diptera (SEKERIS and KARLSON 1966; MCCAMAN, MCCAMAN and LEES 1972; CHEN and HODGETTS 1974).

The studies of KARLSON and co-workers (SEKERIS and KARLSON 1966) and, more recently, those in our laboratory (CHEN and HODGETTS 1974), suggest that the moulting hormone, ecdysone, is involved in the appearance of the activity of this enzyme. Further studies on the regulation of this enzyme and the hormonal control of sclerotization would be greatly facilitated by the introduction of genetical methodology. The attainment of this goal would permit an analysis of the spectrum of mutations that affect the enzyme and this, in turn, might shed some light on the informational complexity which resides in the eukaryotic gene. We report here our first step in this direction: the identification of a small region near the base of chromosome *2L* in *Drosophila melanogaster* which may include the structural gene(s) of dopa decarboxylase.

The method we used to locate this region involved determining whether or not increased enzyme activity could be correlated to increased gene dosage in a particular segment of the Drosophila genome. Variations in enzyme activity in response to a variation in gene dosage have been reported in Drosophila for both *X*-linked genes (STEWART and MERRIAM 1974; SEECOF, KAPLAN and FUTCH 1969; TOBLER, BOWMAN and SIMMONS 1971; BAILLIE and CHOVNICK 1971) and autosomal genes (GRELL 1962; GLASSMAN, KARAM and KELLER 1962; O'BRIEN and GETHMANN 1973). In certain cases the location of the structural gene was known beforehand, and variations in enzyme activity were observed only when the aneuploid segment included the site of the structural gene. This type of approach was successfully employed to assign chromosomal locations to the genes of nine enzymes in the Jimson weed, *Datura stramonium* (CARLSON 1972). An

almost complete set of trisomics is available in this plant and correlations between elevated enzyme activity and trisomy of a particular chromosome were found.

In Drosophila, trisomy for either of the two large autosomes (which together comprise about 80% of the genome) results in lethality. However, aneuploid flies which carry an extra copy of small segments of the genome can be produced. In this study, we analyzed a set of such segmental aneuploids which among them covered virtually the entire genome of the organism. The duplicated regions ranged in size from about 1% to 10% of the genome.

MATERIALS AND METHODS

Production of aneuploids: A genetic technology for producing segmental aneuploids in the two large autosomes of Drosophila was developed in the laboratories of LINDSLEY and SANDLER (LINDSLEY *et al.* 1972) and this has recently been extended to the *X* chromosome (STEWART 1973). The practical details of the procedures are somewhat complicated and can be found in the preceding two references. However, an outline of the methodology we used will be provided here for the purpose of discussion. Figure 1 summarizes the way in which aneuploids carrying

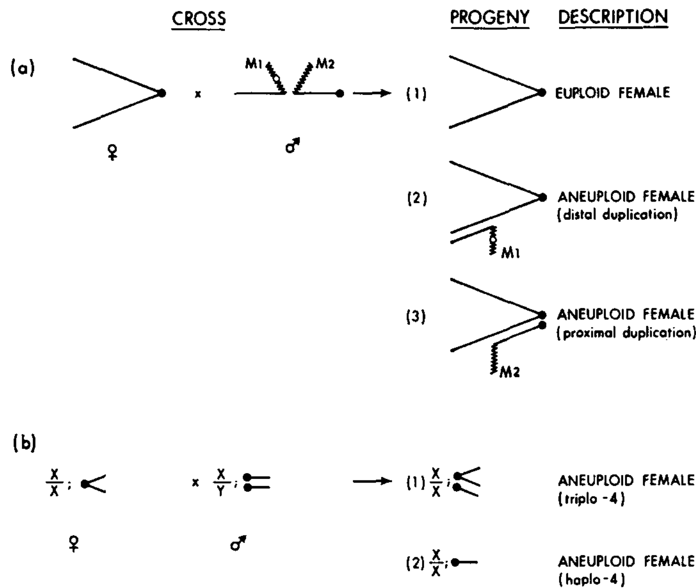

FIGURE 1.—The production of aneuploidy for the *X* and *4*th chromosomes. Only those classes of progeny relevant to the discussion are shown.

(a) Segmental duplications covering the *X* chromosome. Attached-*X* females were crossed to males bearing an *X-Y* translocation. The dominant markers *M1* and *M2* are carried by the *Y* chromosome that was involved in the translocation. (The actual marker genes used were y^+ and B^S). *Y* chromosome material is indicated by ⁓ in both Figures 1 and 2, and the *Y* centromere by an open circle (o).

(b) Duplication of the *4*th chromosome. Genetically marked attached-*4* females were mated to appropriately marked males with free *4*th chromosomes. Triplo-*4* progeny together with a few haplo-*4* individuals were produced. Euploid females with attached *4*th chromosomes were used as controls.

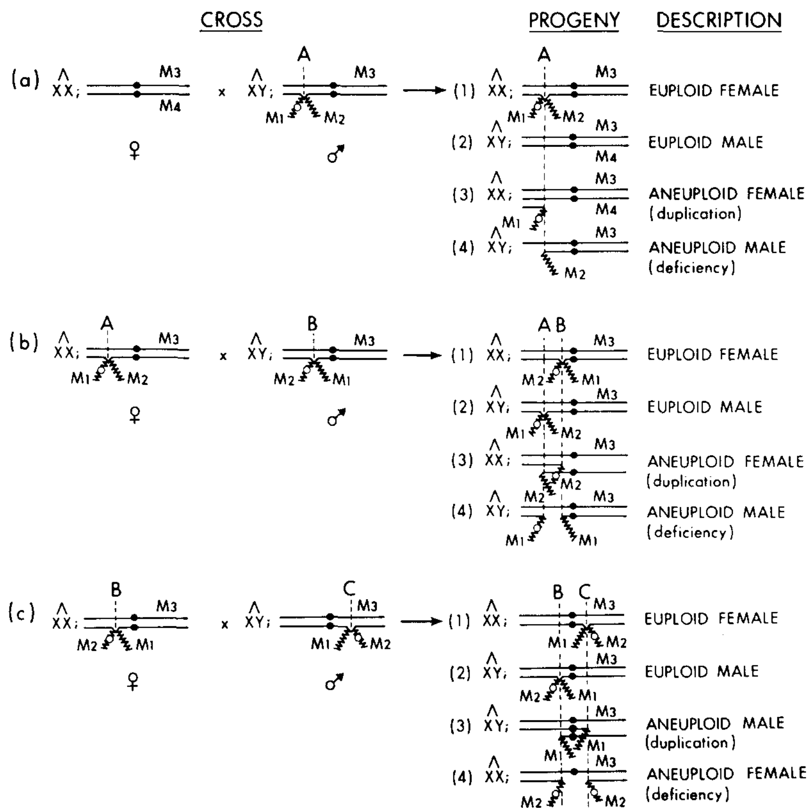

FIGURE 2.—The production of segmental aneuploids on chromosomes *2* and *3*. A hypothetical series of crosses (a), (b) and (c) is shown which illustrates the 3 classes of aneuploidy encountered. Only those classes of progeny relevant to the discussion are included. *XX*—compound attached-*X* chromosome, *XY*—compound attached-XY chromosome.

(a) Terminal aneuploid segment. *M1* and *M2* are the dominant markers carried on the *Y* chromosome that was involved in the *Y*-autosome translocation. The autosomal breakpoint is located at A. Dominant markers *M3* and *M4* facilitate the identification of unbroken chromosomes and obviously differ for the two autosomes. (The actual markers used may be ascertained from the stocks listed in the APPENDIX). Note that the duplication-bearing females (*M1*, *M3*, *M4*) are phenotypically distinguishable from the control females (*M1*, *M3*, *M2*).

(b) Interstitial aneuploid segment, centromere not included. The females used in this cross are from the same stock as the males used in (a). Breakpoints A (distal) and B (proximal) are carried in the female and male parents, respectively, which insures that the duplication-bearing progeny are females. Note again that the euploid controls are phenotypically distinct from their aneuploid sibs of the same sex.

(c) Interstitial aneuploid segment, centromere included. The females used in this cross are from the same stock as the males used in (b). To generate the duplications, an adjacent I segregation must occur in one parent and an adjacent II must occur in the other. Adjacent II segregations are very much less frequent in males than females and as a result, the duplication-bearing aneuploid progeny are males in the crosses which span the centromeres. It can be seen that the sequence of crosses shown above generates a set of contiguous duplications (or deficiencies) independent of the accuracy with which the autosomal breakpoints have been determined.

partial duplications of the X chromosome or a duplication of the *4*th chromosome were obtained. Figure 2 describes the crosses in which segmental aneuploids on chromosomes *2* and *3* were obtained. The technique involved crossing two parents each heterozygous for different translocations between a genetically marked Y chromosome and the autosome. With the exception of the two crosses involving parents with breakpoints on opposite sides of the centromere (Figure 2c), crosses were always set up so that the partially trisomic progeny were females and the partially monosomic progeny were males. The autosomal breakpoints in the parents were generally so far apart that although the hyperploid progeny survived, the hypoploid progeny did not. In the appropriate crosses between 12 stocks, we produced a set of segmental aneuploids bearing a series of non-overlapping, contiguous duplications which covered the entire second chromosome. An analogous set of aneuploids bearing duplications which covered the entire third chromosome with the exception of a region between 82C and 83EF was produced in a series of 14 crosses. In this case, two of the duplicated regions overlapped one another. The stocks used for the above crosses are listed in the APPENDIX.

Enzyme assay: Measurements of dopa decarboxylase activity were carried out as described elsewhere (HODGETTS and KONOPKA 1973). Generally 4–7 unfrozen adults, 4–8 hours old were homogenized in 0.27 ml of 0.05 M Tris, 10^{-3} M phenylthiourea buffer (pH 7.4 at 22°). The $^{14}CO_2$ which was released from [1-^{14}C] dopa by enzymatic activity in a reaction chamber suspended in a scintillation vial over a carbon dioxide trapping agent, was detected by liquid scintillation counting using Bray's solution. Duplicate extracts were made, and enzyme assays were carried out in triplicate on each extract. Enzyme activity in the aneuploid progeny was then compared to that in the sibling euploid progeny of the same sex.

RESULTS

With the exception of the small region (82C to 83EF) near the centromere on chromosome *3R*, which includes a triplo-lethal locus (LINDSLEY *et al.* 1972), flies carrying duplications of the entire chromosome complement were analyzed. As the data in Figure 3 illustrate, a 64% increase in enzyme activity was found in aneuploid flies carrying a duplication of the region 34B to 39C on chromosome

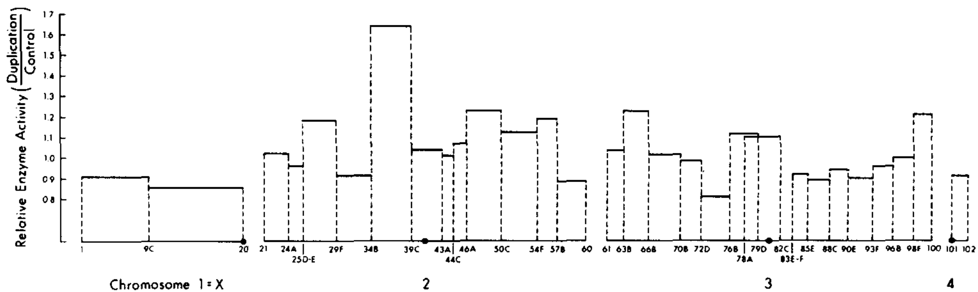

FIGURE 3.—The response of dopa decarboxylase activity to aneuploidy in the Drosophila genome.

Crosses were made according to the conventions outlined in Figures 1 and 2, using the stocks listed in the APPENDIX. The ratio of enzyme activity in duplication-bearing flies to that in euploid controls is shown for the set of 29 duplications that we used. For each region, the average of all the determinations is shown and includes at least two independent replicates in every case. The duplications are contiguous except in the region between 82C and 83EF and non-overlapping except in the region 78A–79D. The extent of each duplication is indicated by conventional salivary chromosome reference numbers (LINDSLEY and GRELL (1968).

2. An increase near the expected value of 50% was not observed in any other region of the genome.

Further experiments were performed to localize the dosage-sensitive locus within the region 34B–39C and the results are summarised in Figure 4, experiments 2–4. The region was successively subdivided, by sets of continguous non-overlapping duplications. At each successive stage, increased enzyme activity was associated with only one segment, and thus an unambiguous localization of the dosage-sensitive region could be made. As shown in experiment 4, this region was between 36EF and 37D, which are the autosomal breakpoints of stocks D219 and H174, respectively.

In every cross in experiments 1–4 of Figure 4, in which a dosage effect was

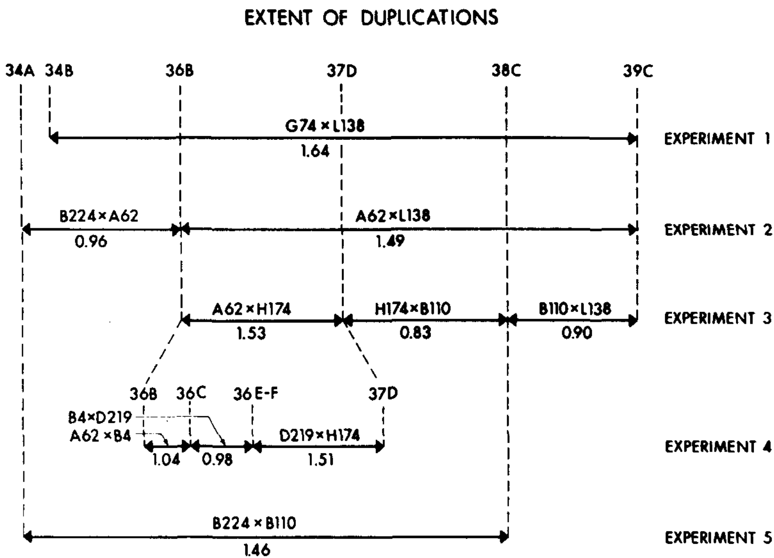

FIGURE 4.—The response of dopa decarboxylase activity to aneuploidy in the region 34A–39C. The stock numbers used for each cross are indicated above the line showing the extent of the aneuploid segment and have been described elsewhere (LINDSLEY *et al.* 1972). Females from the first stock were mated to males from the second stock, in accordance with the scheme in Figure 2b. Dopa decarboxylase activity in the duplication-bearing females was compared to the activity in their euploid sisters. The ratio of the enzyme activity in the aneuploids to that in the euploids is given below the line, for each interstitial segment analyzed.

To determine whether or not the (duplication/control) ratio for the region 36EF to 37D differed significantly from that of the other duplications covering the remainder of the genome (experiments 2, 3 and 4 and Figure 3), a one-way analysis of variance and Scheffé multiple contrasts were performed. All the available data for the region 36EF to 37D (including those in Tables 2 and 3) were used for these analyses. The one-way analysis of variance showed that the (duplication/control) ratios were not all equal ($F_{33,68} = 4.43$; $p < 0.001$). Using a Scheffé multiple contrast, the hypothesis that the 36EF to 37D (duplication/control) ratio was 1.5 times the average (duplication/control) ratio for the remainder of the genome was accepted. Furthermore, it was possible to reject the hypothesis ($p < 0.001$) that the ratio (duplication/control) for 36EF to 37D was equal to the average (duplication/control) ratio for the remainder of the genome (the value for the Scheffé contrast in this case was $S = 9.13$).

detected, the duplication-bearing progeny were of yellow phenotype, whereas the control flies had a normal body color. During our screening of the genome we had not observed any abnormal enzyme activity in those crosses which produced yellow aneuploid progeny. However, to rule out that the increased enzyme activity we observed in the aneuploids in experiment 1–4 was attributable to the yellow phenotype, we set up the cross shown in experiment 5 (Figure 4). In this case both the aneuploid and euploid progeny had normal body coloring and we observed a 46% increase in enzyme activity in the aneuploid over the control progeny.

The region 36EF to 37D is sufficiently small that aneuploids carrying both duplications and deficiencies were recovered, although the viability of the deficiency-bearing flies was only 15% that of the control flies. It was therefore possible to examine the change in the enzyme activity in response to a 3-fold variation in the gene dosage. As the data in Table 1 show, enzyme activity was well correlated to the number of copies of this region. Predicted activities were obtained for 1, 2 and 3 gene doses by linear regression analysis of this data. The ratios for the aneuploids relative to the diploid were 14% below 0.5 for one dose and 5% above 1.5 for three doses. These discrepancies probably stem from the relatively poor health of the hypoploids. As mentioned above, these had only 15% of the viability of the euploids, whereas the hyperploids had 79% of the viability of the euploids. The simplest conclusion one can draw from these observations is that the structural gene for dopa decarboxylase is included in the region between 36EF and 37D.

It might be argued that a duplication of the region 36EF to 37D causes a general elevation in the activity of many enzymes on chromosome *2*. However, this does not appear to be the case. O'Brien and Gethmann (1973) have shown that flies bearing a duplication for the region 35BC to 38C (which includes 36EF to 37D) possess normal levels of both α-glycerolphosphate dehydrogenase (whose structural gene lies between 25F and 26B) and alcohol dehydrogenase, whose structural gene is located between 35A1 and 35B5 (Nash 1970).

TABLE 1

*Dopa decarboxylase activity in flies with one, two and three copies of the region 36EF to 37D**

	Dopa decarboxylase activity (n moles/min/mg live weight) ($\times 10^2$)			
Dosage	Experiment 1	Experiment 2	Average	Observed relative activity
3 copies	1.61	1.71	1.66	1.50
2 copies	0.93	1.23	1.08	1.00
1 copy	0.39	0.48	0.44	0.41

* The flies were 3–10 hours old and were obtained from the cross D219 (♀) × H174 (♂), in accordance with the scheme shown in Figure 2b.

The separate linear regression equations for the two experiments of enzyme activity as a function of gene dosage had identical slopes. This justified fitting the data with a common regression equation which was: $A(x) = 0.607x - 0.152$, where $A(x)$ = enzyme activity ($\times 10^2$) and x = the number of copies assumes values of 1, 2 or 3. The 95% confidence interval of the slope was 0.607 ± 0.14 and this regression accounted for 85% of the variance.

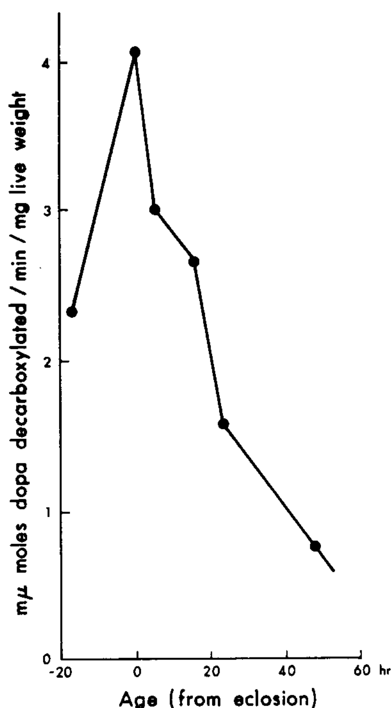

FIGURE 5.—The variation of dopa decarboxylase activity with age in wild-type (Canton-S) flies. The values represent the average of two determinations.

The activity of dopa decarboxylase changes rapidly during the first day following eclosion of the adult (Figure 5). A slight shift in the time at which the enzyme activity reached its maximum value in either the aneuploids or the euploids is conceivable. This could produce the relative increase in activity that we observed in the aneuploids. Alternatively, age differences in the small samples used might be invoked to explain the increased enzyme activity in the aneuploids. As a final control to eliminate these possibilities, progeny of different ages from the cross between stocks D219 and H174 were analyzed. As Table 2 shows, a consistent increase in enzyme activity was found for the aneuploids throughout the first day of adult life.

DISCUSSION

In view of our conclusion that the structural gene for dopa carboxylase lies in the region 36EF to 37D, it is of considerable interest that mutants with increased sensitivity to α-methyl dopa, an inhibitor of dopa decarboxylase, have been mapped to position 53.1 $\pm$ 0.5 on the left arm of chromosome *2* (SPARROW and WRIGHT 1974). This site is very close to the region 36EF to 37D and may be included within it. The mutants sensitive to α-methyl dopa have normal levels of dopa decarboxylase, so it is not possible to conclude that the gene for this enzyme has mutated. However, in the light of our study we feel that this is a

TABLE 2

*Dopa decarboxylase activity in euploid and duplication-bearing females at various times during the first day following eclosion**

Age (hrs after eclosion)	Dopa decarboxylase activity (n moles/min/mg live weight) (× 10^2)		Ratio of activities $\left(\frac{\text{duplication}}{\text{control}}\right)$
	Duplication	Control	
¼–4¼	2.89	1.96	1.47
4¼–7¼	2.63	1.74	1.51
18–20	1.89	1.28	1.48

* The flies were obtained from the cross D219 (♀) × H174 (♂), which produces aneuploidy for the region 36EF to 37D.

strong possibility. We now are attempting to isolate small deficiencies in the region 36EF to 37D that should clarify the point.

In the present study, the absence of a procedure for visualizing the activity of dopa decarboxylase on an electrophoretic support medium precluded the use of isozymes to locate the structural gene (see, for example, YOUNG 1966). Likewise, the requirement for a purified transcription product prevented the use of autoradiography to identify the site of hybridization on the salivary chromosomes of a specific messenger RNA, as has been done for the histones in Drosophila (BIRNSTIEL, WEINBERG and PARDUE 1973). In this particular situation, the systematic variation of gene dosage as a means for identifying the structural gene was a useful alternative. While undoubtedly subject to difficulties in certain cases (O'BRIEN and GETHMANN 1973), this method nevertheless promises to play an important role in the definition of new gene-enzyme systems in Drosophila.

I wish to thank LARRY SANDLER who graciously provided me with the opportunity of iniating this work in his laboratory. I am further indebted to both ADELAIDE CARPENTER and BRUCE BAKER for their encouragement and patient clarifications, and to KEN MORGAN for the statistical analyses. Finally, it is a pleasure to acknowledge the assistance of JIM BERERTON throughout this work. A grant (No. A6477) from the National Research Council of Canada supported this research.

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Corresponding editor: D. T. SUZUKI

APPENDIX

The stocks that were used to generate the segmental duplications presented in Figure 2 are listed below

	Stock designation	Breakpoints	Source
X Chromosome	T(X;Y) B26	9C; Y^S	J. R. MERRIAM
Chromosome *2*	*C(1)RM,y;Sco/Bal**	—	L. SANDLER
	T(Y;2) G120	24A; Y^S	L. SANDLER
	T(Y;2) B236	25DE; Y^L	L. SANDLER
	T(Y;2) A145	29F; Y^S	L. SANDLER
	T(Y;2) G74	34B; Y^L	L. SANDLER
	T(Y;2) L138	39C; Y^S	L. SANDLER
	T(Y;2) J59	43A; Y^S	L. SANDLER
	T(Y;2) H136	44C; Y^L	L. SANDLER

	T(Y;2) A24	46A; Y^S	L. Sandler
	T(Y;2) L110	50C; Y^L	L. Sandler
	T(Y;2) H149	54F; Y^S	L. Sandler
	T(Y;2) L107	57B; Y^L	L. Sandler
Chromosome *3*	*C(1)RM,y;Sb/Bal*†	—	L. Sandler
	T(Y;3) A14	63B; Y^L	L. Sandler
	T(Y;3) H138	66B; Y^S	L. Sandler
	T(Y;3) R83	70BC; Y^L	L. Sandler
	T(Y;3) B207	72D; Y^S	L. Sandler
	T(Y;3) L14	76B; Y^L	L. Sandler
	T(Y;3) J162	79D; Y^S	L. Sandler
	T(Y;3) R153	78A; Y^L	L. Sandler
	T(Y;3) A154	82C; Y^L	L. Sandler
	T(Y;3) L136	83EF; Y^S	L. Sandler
	T(Y;3) G42	85E; Y^L	L. Sandler
	T(Y;3) G48	88C; Y^S	L. Sandler
	T(Y;3) B116	90E; Y^L	L. Sandler
	T(Y;3) B93	93F-94A; Y^S	L. Sandler
	T(Y;3) B197	96B; Y^L	L. Sandler
	T(Y;3) A82	98F; Y^S	L. Sandler
Chromosome *4*	spa^{pol}	—	L. Sandler
	$C(1)RM,ci\ ey^R$	—	L. Sandler

* *Bal* represents either $In(2L+2R)Cy, Cycn^2$ or $In(2LR)SM1, al^2Cycn^2sp^2$ (Lindsley et al. 1972).

† *Bal* represents $In(3LR)TM6, ss^{-}bx^{34e}Ubx^{67b}e$.