Maroni, G. University of North Carolina, Chapel Hill, North Carolina. A duplication of Adh in association with Sco.

From the following cross: b el Adh^{n1}/Adh^{F}Sco X b el Adh^{n1}/b el Adh^{n1} two recombinants carrying b el Sco among a total progeny of 10,094 flies were obtained. Both were Adh-positive. One of these chromosomes was kept in a stock and further analyzed by genetic means. As a result of these studies, it is concluded that this recombinant chromosome carries a duplication that involves the Adh locus and that this duplication may have arisen as a consequence of the Sco mutation being associated with a chromosomal rearrangement. The approximate map positions of the loci used are shown below (O'Donnell et al. 1977 and pers. obs.). 1^2 is a lethal mutation obtained in our laboratory by treatment with EMS. The remaining markers are described in Lindsley and Grell (1968). Several crosses were performed to select recombinants between 1^2 and Sco. Heterozygotes of the type 1^2el+Sco+/1^{2+}el Sco were crossed to males carrying the deletion Df (2L) 64j, pr which includes both loci. The specific genotypes used in the different experiments and the results obtained are indicated in Table 1.

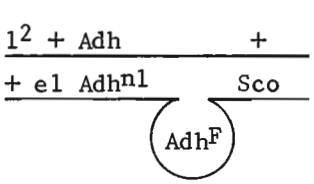
b	1^2	el	Adh	Sco
48.5	49.8	49.9	50.1	50.12

Table 1

Parents			Progeny		
Female	X	Male	Curly	$b1^{2+}el^{+}Sco^{+}$	$b1^{2+}el$ Sco^{+}
$\frac{b\ 1^{2+}\ Adh^{B7}+}{b\ +\ el\ Adh^{F}Sco}$			899	1	8
$\frac{+\ 1^{2+}\ Adh^{A1}+}{b\ +\ el\ Adh^{F}Sco}$		$\frac{Df(2L)64j,pr}{Cy0}$	1287	0	4
$\frac{+\ 1^{2+}\ Adh^{n5}+}{b\ +\ el\ Adh^{F}Sco}$			4707	16	33
Total			6893	17	45

Recombinants were crossed to a second-chromosome balancer stock for further tests. Of the 45 recombinant chromosomes between el and Sco, three had detectable ADH activity and its electrophoretic mobility corresponded to the chromosome carrying 1^2: in two cases Adh^{n5} and in the other case Adh^{B7}. The remaining 42 recombinants had no ADH activity at all. When these ADH-negative chromosomes were made heterozygous with an ADH-slow allele and extracts run in polyacrylamide gel electrophoresis, staining of the gels for ADH put in evidence a band of activity running slightly ahead of the major ADH-S band. This behavior is typical of the Adh^{n1} allele and it is due to the fact that the inactive polypeptide made by Adh^{n1} produces an active heterodimer with the ADH-S polypeptide.

Since this result was obtained with recombinants from three different Sco^{+} chromosomes and since neither Adh^{B7} nor Adh^{A1}, both with slow mobility, show by themselves the extra band indicating the presence of an inactive polypeptide, the presumed Adh^{n1} allele must be carried by the b el Sco chromosome. Given the way in which this chromosome was obtained (see above), it seems reasonable to suspect that unequal crossing-over occurred leading to the production of a duplication-carrying chromosome: b el $Adh^{n1}Adh^{F}$ Sco such that pairing with it and cross ing over would occur as follows:



If this were the case, it should be possible to recover, also, the reciprocal cross-over class with the Adh allele of the Sco^{+} chromosome and Adh^{F} from the Sco chromosome. In the crosses presented above, such flies do not survive because 1^2 and Sco are uncovered by the deficiency 64j.

Crosses were then set up in which recombination between el and Sco would be detected by visual inspection, allowing the survival of all classes of progeny. The results obtained are as follows:

Parents			Progeny		
Female	X	Male	Parental	1^2el$^+$Sco	1^{2+}el Sco$^+$
$\frac{+\ 1^2\ +\ Adh^{Al}\quad +}{b\ +\ el\ Adh^{nl}Adh^{F}Sco}$			1235	8	7
$\frac{+\ +\ Adh^{S}\quad +}{b\ el\ Adh^{nl}Adh^{F}Sco}$	X	$\frac{b\ el\ Adh^{nl}}{b\ el\ Adh^{nl}}$	3604	15	13
Total			4839	23	20

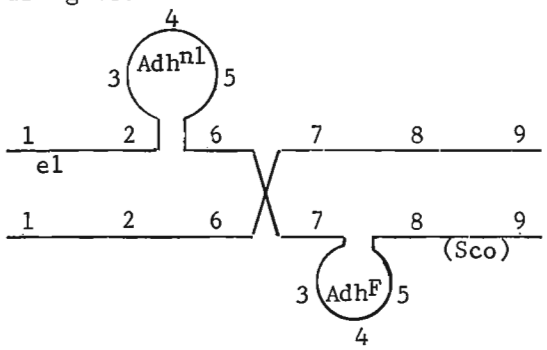
The 23 1^2el$^+$Sco chromosomes were made heterozygous with CyO Adh^n, a balancer chromosome that produces no detectable ADH polypeptide. Seventeen of them produced the multiple bands characteristic of flies with two active alleles, one slow and one fast, as in heterozygotes. In these cases the two alleles are in the same chromosome indicating the existence of a duplication. The remaining five recombinant chromosomes were phenotypically ADH-F. Whether Adh^{nl} is present or not in these chromosomes has not been established.

Of the 20 recombinants 1^{2+}el Sco+, as before, the majority (16) were ADH-negatives and upon testing in heterozygotes with ADH-slow they put in evidence the presence of an Adh^{nl}-like allele. Of the remaining four, one had Adh^{Al} and three Adh^S without Adh^{nl}. These results lend support to the interpretation given in the diagram shown above. That is to say that in b el Sco there is an Adh^F gene, presumably the one in the original Sco, that is positioned to the right of the normal Adh site and another Adh gene which occupies the standard Adh locus, as determined by the fact that the specific allele present at this second site can be interchanged by crossing over with a standard-sequence chromosome.

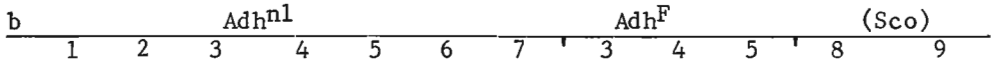
One question of interest is to establish whether the occurrence of a duplication in b el Sco has a structural basis in the two chromosomes originally involved: b el Adh^{nl} and Sco. It has been suggested by Ashburner, Woodruff and Camfield (pers. comm.) that Sco is a rearrangement involving at least three breakpoints to the right and left of Adh. This suggestion is based on the properties of reversions of Sco and cytological observations. A deletion-insertion type of rearrangement with breakpoints as indicated in the figure below would fit the observations presented here.

$$\frac{el^+\ \ Adh^+}{1\ 2\ 3\ 4\ 5\ 6\ 7\ 8\ 9} \longrightarrow \frac{el^{+(Sco)}\ \ Adh^+}{1\ 2'6\ 7'3\ 4\ 5'8\ 9}$$

During recombination with a normal chromosome:



To give rise to the b el Sco chromosome, with the Adh^{nl}, Adh^F duplication.



An attempt was made to test this interpretation by generating new duplications and the expected reciprocal deletions. Heterozygotes b el Adh^{F}rd pr cn/Adh^{F}Sco were crossed to males b el Adh^{nl} to detect recombination between el and Sco. Recombinants b el Sco would carry two doses of Adh^F and show a marked increase in ADH activity and, more conclusively, recombinants b$^+$el$^+$rd pr cn would be deficient for ADH. The result is shown in the table below.

Of the four recombinants between el and Sco three were b$^+$el$^+$rd pr cn and all of them had Adh^F. One was the reciprocal, b el Sco; it could not be kept in a stock but the recombinant itself did not seem to have an increased level of ADH. Thus, these results are inconclusive in demonstrating that Sco is a transposition. It is entirely possible that the size of the segments involved

Parents			Progeny		
Female	X	Male	Parental	cross-over between b and el	cross-over between el and Sco
$\frac{b\ el\ Adh^{F}rd\ pr\ cn}{+\ +\ Adh^{F}\quad +\ (Sco)}$	X	$\frac{b\ el\ Adh^{nl}}{b\ el\ Adh^{nl}}$	6711	120	4

and constraints on the recombinational process imposed by the rearrangement itself make recombination in the segment 6-7 much less frequent than in the segment 1-2 (see diagram).

The frequency of recombination in this region also supports the idea that b el Sco carries a sizeable duplication. The frequency of recombination between el and Sco in this chromosome is 1.3% in the experiments in Table 1 and 0.9% in those in Table 2. The recombination frequency between these two markers when the original Sco chromosome is involved is 0.06% from Table 3, a value which is in agreement with published results. It should be mentioned that in all the crosses described here females also carried a pair of attached X's (C(1) RM, y). The results presented here, although they do not close the issue, fit well with the idea that Sco is a multiple point rearrangement. Possibly an insertion of a chromosomal segment, which includes Adh, to a position slightly to the right of its normal location. It might be noted that if this is correct the left breakpoint of this segment (2-3 in the diagrams) should be to the right of el since the recombinant b el Sco carries Adh^F in the duplicated piece (the insertion) but not el^+. Finally, the chromosome b el Sco is a useful tool to generate duplications involving any allele of Adh in combination with Adh^F.

References: Lindsley and Grell 1968, Carnegie Inst. Wash. Publ. 627; O'Donnell, J. et al. 1977, Genetics 86:553-566.

Maróy, P., K. Koczka, É. Fekete and J. Vargha. Biological Research Center, Szeged, Hungary. Molting hormone titer of D. melanogaster larvae.

The molting hormone (MH) titer of D. melanogaster has been studied during metamorphosis by Borst and O'Connor (1972), de Reggi et al. (1975), and Hodgetts et al. (1977). In this paper we study the changes of MH titer during larval life of D. melanogaster using MH specific radioimmunoassay (Maróy et al. 1977). Eggs were collected for a period of one hour and cultures were synchronized for hatching. Specimens were weighed and homogenized in an all-glass Potter-type homogenizer in 60% methanol, and treated in the standard way according to Maróy and Tarnóy (1978).

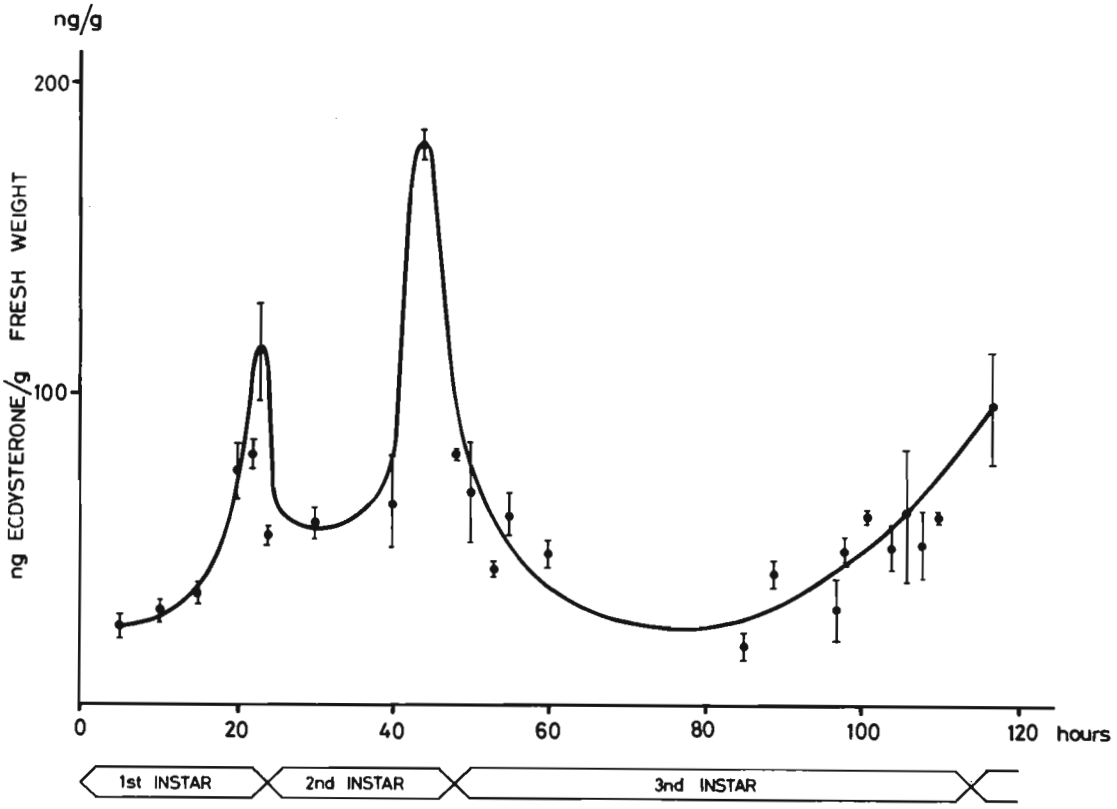


Fig. 1. MH titer of D. melanogaster during larval life.