The EMBO Journal vol.4 no.1 pp.163 – 166, 1985

The hierarchical relation between X-chromosomes and autosomal sex determining genes in *Drosophila*

Monica Steinmann-Zwicky and Rolf Nöthiger

Zoological Institute, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Communicated by R. Nöthiger

The classical balance concept of sex determination in *Drosophila* states that the X-chromosome carries dispersed female-determining factors. Besides, a number of autosomal genes are known that, when mutant, transform chromosomal females (XX) into pseudomales (*tra*), or intersexes (*ix, dsx, dsx^D*). To test whether large duplications of the X-chromosome have a feminizing effect on the sexual phenotype of these mutants, we constructed flies that were mutant for *ix, dsx, dsx^D* or *tra* and had two X-chromosomes plus either a distal or a proximal half of an X-chromosome. These or even smaller X-chromosomal fragments had a strong feminizing effect when added to triploid intersexes (XX; AAA). In the mutants, however, no shift towards femaleness was apparent. We conclude that enhancing the female determining signal is ineffective in flies that are mutant for an autosomal sex determining gene, and therefore, that these genes are under hierarchical control of the signal given by the X:A ratio. Parallels between sex-determining and homeotic genes are drawn.

Key words: Drosophila/sex determination/X-chromosome/sex determining genes/genetic hierarchy

Introduction

In *Drosophila*, sex is determined by the ratio of X-chromosomes (X) to sets of autosomes (A). An X:A ratio of 1.0 (XX; AA) results in female development, a ratio of 0.5 (X; AA) in male development. The Y-chromosome plays no role in sex determination. A mosaic of male and female structures is produced in triploid intersexes with a ratio of 0.67 (XX; AAA, triploid flies with only two X-chromosomes). These results led Bridges (1921,1925) to conclude that the X-chromosome carries female determinants whereas the autosomes harbor male determinants. He formulated his classical theory of genic balance to explain sex determination in *Drosophila*. Sex was visualized as a quantitative character with continuous variation, brought about by the balance between female and male determining genetic factors.

Later, a small number of autosomal genes were discovered that, when mutant, transform XX-flies into sterile pseudomales, e.g., transformer (*tra*; Sturtevant, 1945), transformer-2 (*tra-2*; Watanabe, 1975), or into intersexes, e.g., intersex (*ix*; Morgan *et al.*, 1943), double sex (*dsx*; Hildreth, 1965). At the *dsx*-locus, there also exists a dominant allele, *dsx^D*, that produces intersexes when heterozygous (*X/X; dsx^D/+*), or pseudomales in combination with *dsx* (*X/X; dsx^D/dsx*) (Duncan and Kaufman, 1975). These autosomal genes play an important regulatory role in sex determination (Baker and Ridge, 1980; for review, see Baker and Belote, 1983).

The question arises how addition of X-chromosome material, which carries female determinants, will affect the sexual phenotype of intersexes or pseudomales. Dobzhansky and Schultz (1934) tested the effects of various portions of the X-chromosome on sex determination in triploid intersexes (XX; AAA). When they added a duplication (Dp) of part of an X-chromosome (XX + Dp; AAA), the sexual development was shifted towards femaleness, whereas deleting part of an X-chromosome resulted in a shift towards maleness. The shift was proportional to the size of the duplication or deletion, and largely independent of the region of the X that was duplicated or deleted. The authors concluded that female determinants are scattered over the entire X-chromosome.

Our aim was to test whether large duplications of the X-chromosome could also feminize the sexual phenotype of diploid XX-flies that were mutant for one of the autosomal sex determining genes. A shift towards femaleness is expected if the X:A ratio, beside its regulatory effect on the sex determining genes *ix, tra* and *dsx*, can also directly influence the sexual differentiation genes, i.e., the genes that are involved in the differentiation of the sexual characters. Such a conclusion was reached by Sturtevant (1945) for *Drosophila*, and was also considered by Hodgkin (1983) for the nematode *Caenorhabditis* (see Results and Discussion). If, however, the X:A ratio is the primary genetic signal that regulates the autosomal sex-determining genes in a strictly hierarchical pathway, then duplications of the X-chromosome will have no feminizing effect on the sexual phenotype of flies that are mutant for *ix, tra* or *dsx*. As a test we constructed and analyzed flies that were mutant for one of the autosomal sex-determining genes and had two X-chromosomes plus either a distal or a proximal half of an X-chromosome (Figure 1).

Results and Discussion

The results from the analysis of the genitalia are graphically presented in Figure 2 (see legend for explanation). We must point out that in *Drosophila* both sexes have a male and a female genital primordium of which normally only one differentiates genital structures whereas the other remains developmentally repressed (Nöthiger *et al.*, 1977; Schüpbach *et al.*, 1978). In intersexual flies, both primordia develop to a certain extent so that one fly may display male and female genital structures (Epper, 1981; Epper and Nöthiger, 1982). The graph shows that addition of a proximal half (X^P) or distal half (X^D) of an X-chromosome had no feminizing effect in any of the tested genotypes. Rather, addition of half an X resulted in a reduction of female structures in all genotypes that cause intersexual development.

No effect was seen in pseudomales, *tra/tra* and *dsx^D/dsx*, except that in the latter genotype the penis apparatus was slightly reduced when X^P was added, and a rudimentary seventh tergite (T7) appeared when X^D was added. The T7 is a structure that displays sexual dimorphism: it is absent in males and pseudomales, and is always present in females and

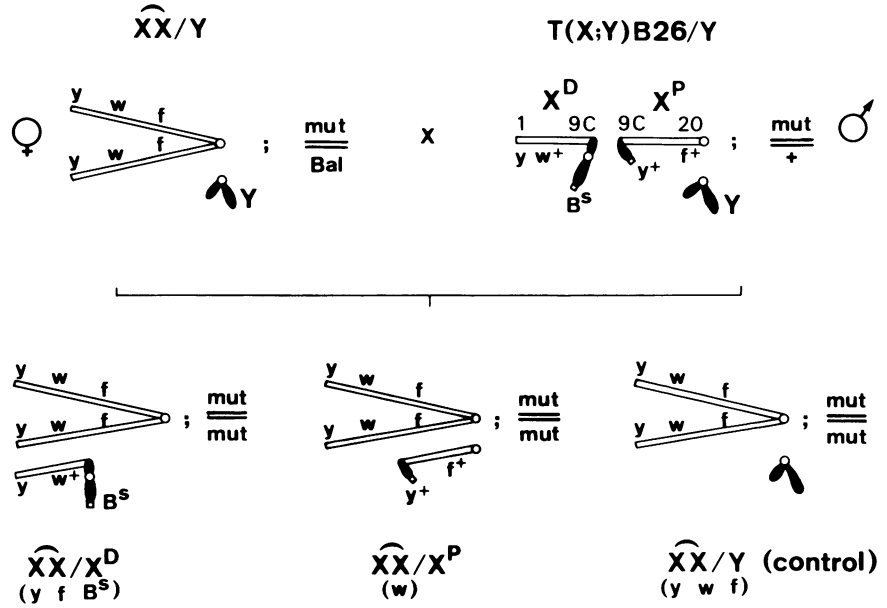

Fig. 1. Construction of flies being mutant at an autosomal sex-determining locus (*ix, tra, dsx*), and carrying 2½ X chromosomes. The female parent had attached-X chromosomes (XX) and was heterozygous for *ix, tra,* or *dsx* (symbol *mut*) and a balancer chromosome (*Bal*). The male parent carried a reciprocal translocation between X and Y, T(X; Y)B26, plus a free Y, and was also heterozygous for a mutation at one of the autosomal sex determining loci (symbol *mut*). The translocation breaks the X chromosome at 9C, i.e., approximately in the middle of the euchromatin (Stewart and Merriam, 1975). Segregation in the parents leads, among others, to the three genotypes that were analyzed for their sexual phenotype. The aneuploid genotypes carried two complete X-chromosomes (XX) plus either a distal half (X^D) or a proximal half (X^P) of an X, and were compared with their control sibs (XX/Y). All chromosomes were genetically labelled so that the three desired genotypes were recognizable by cuticular markers independently of their sexual phenotype (for genetic symbols see Lindsley and Grell, 1968). For some genotypes, notably those with *ix, dsx^D* and *dsx*, most of the aneuploid flies did not emerge and had to be dissected out of the puparium.

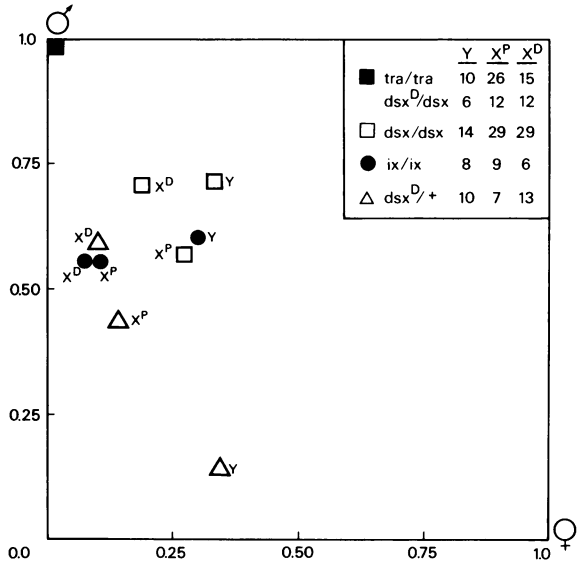

Fig. 2. Graphical representation of the results for the genitalia. The external genitalia of each fly were examined under a compound microscope (200–400 x). For this analysis, the whole fly was mounted under coverslips in Faure's solution. Each fly was assigned two values between O and 1, one for the male and one for the female genitalia. These values were expressed as a fraction of a full set of normal male or female genitalia. Pure females were thus characterized by the coordinates 0/1 (lower right corner, ♀); pure males by 1/0 (upper left corner, ♂); intersexual flies in which various proportions of male and female genitalia were differentiated fell somewhere in between. Thus, each fly occupies a defined place within the coordinate system. The values were assigned according to the size of chitinized genital structures shown in Figure 3. For details of the evaluation scheme see Janning *et al.* (1983). The symbols (■ □ ● △) mark the mean values of a genotype, with (X^P or X^D) or without (Y) an additional half X chromosome. The table in the upper right corner gives the genotype for each symbol and the number of flies whose genitalia were analyzed. Y, stands for XX/Y (controls); X^P for XX/X^P, and X^D, XX/X^D. See Figure 1.

intersexual flies. In this respect, the consistent appearance of a small T7 in *XX/X^D; dsx^D/dsx* may represent a female tendency, and it may mean that the strong masculinizing genetic signal of *dsx^D/dsx* can somehow be weakened or bypassed by feminizing factors on the distal portion of the X-chromosome. Such a possibility was considered by Hodgkin (1983) for *Caenorhabditis elegans*, a nematode whose mechanism of sex determination is strikingly similar to that of *Drosophila*. In *Caenorhabditis*, the mutation *tra-1* transforms XX individuals into pseudomales. As in *Drosophila*, homozygous *tra-1* animals, even with three X-chromosomes, were males, but some male-specific tail structures had disappeared. However, as Hodgkin (1983) also discusses, this phenotype, rather than signalling a partial feminization, could also be the result of a non-specific developmental disturbance caused by the aneuploid genotype. In *Drosophila*, for example, a rudimentary seventh tergite appears in XY males that are mutant for *Abd-B*, a gene in the bithorax complex that has clearly nothing to do with sex determination (Sanchez-Herrero *et al.*, 1984).

The sexual pnenotype of the analia and the sex comb remained essentially the same in all genotypes, intersexes and pseudomales. With X^P, the *anal plates* of intersexual flies (*ix/ix, dsx/dsx, dsx^D/+*) were laterally positioned (Figure 3b) as in the controls (Figure 3a); with X^D they usually were connected dorsally which may represent a female tendency (Figure 3c). The number of *sex comb* bristles was unaltered when X^P was added, but was reduced with X^D (Figure 3d–f). Sturtevant (1945) described a single triplo-X animal that was homozygous for *tra* (*XX/X; tra/tra*). This fly was essentially male, with a typical sex comb that, however, had fewer bristles than in a regular diploid pseudomale (*X/X; tra/tra*). He interpreted this reduction in number of sex comb bristles as a shift towards femaleness and concluded that 3X

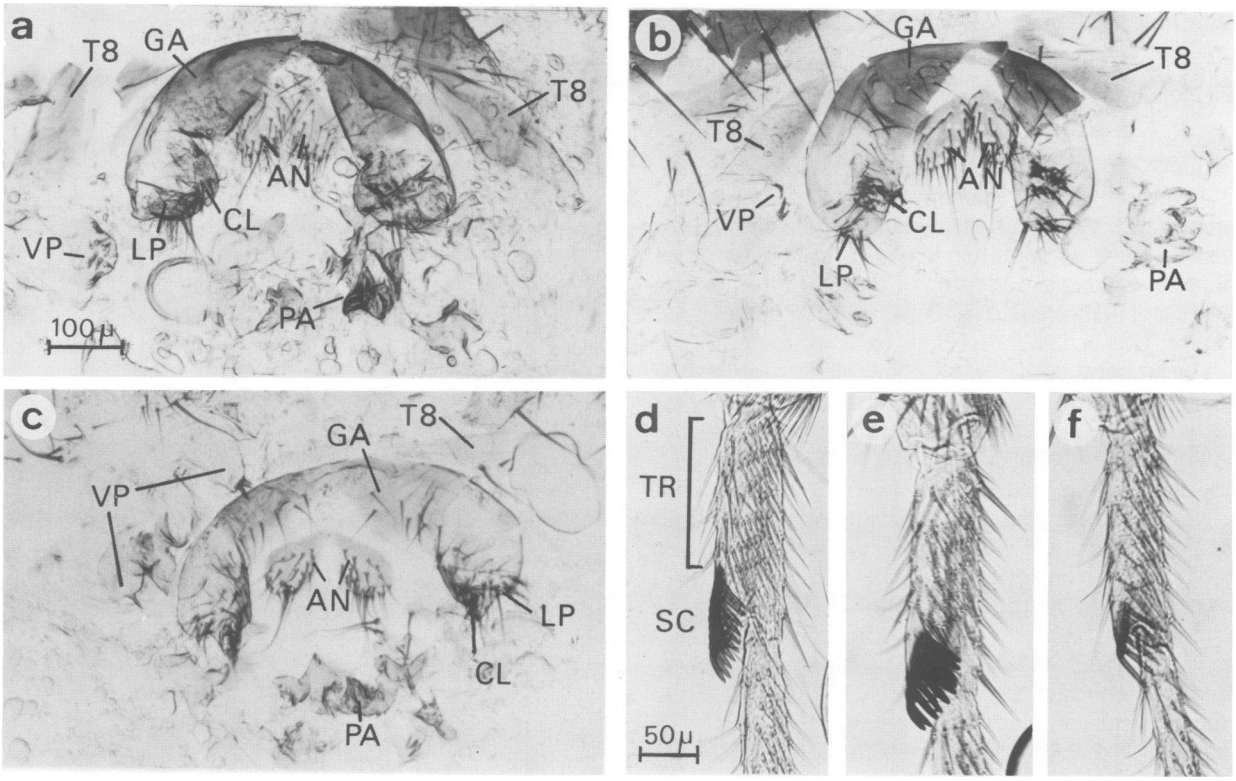

Fig. 3. Photographs of microscopical preparations of external genitalia and analia **(a, b, c)** and of basitarsi **(d, e, f)**. **(a)** $\overline{XX}$/Y; dsx/dsx, **(b)** $\overline{XX}/X^P$; dsx/dsx, **(c)** $\overline{XX}/X^D$; dsx/dsx, **(d)** $\overline{XX}$/Y; tra/tra, **(e)** $\overline{XX}/X^P$; tra/tra, **(f)** $\overline{XX}/X^D$ tra/tra. AN, anal plates. Male genitalia: CL, clasper; GA, genital arch; LP, lateral plates; PA, penis apparatus. Female genitalia: VP, vaginal plates; T8, eighth tergite. SC, sex comb; TR, transversal rows. Most structures, especially the female elements, are severely reduced compared with normal male and female genitalia.

Table I. Number of bristles on basitarsus of forelegs (mean ± S.D.)

Genotype[a]	*n*	Bristles in sex comb[b]	Bristles in transversal rows
$\overline{XX}$/Y; ix/ix	16	6.7±0.7	57.2±5.4
$\overline{XX}/X^D$; ix/ix	13	4.4±0.8	42.7±2.8
$\overline{XX}$/Y; dsx/dsx	28	6.3±0.6	55.2±4.4
$\overline{XX}/X^D$; dsx/dsx	58	4.7±0.8	44.3±6.6
$\overline{XX}$/Y; dsx^D/+	20	6.8±1.5	51.5±4.5
$\overline{XX}/X^D$; dsx^D/+	22	4.3±1.0	30.3±4.2
$\overline{XX}$/Y; dsx^D/dsx	12	8.6±0.6	54.2±1.7
$\overline{XX}/X^D$; dsx^D/dsx	24	5.6±0.9	41.7±3.3
$\overline{XX}$/Y; tra/tra	20	10.2±1.0	59.2±3.0
$\overline{XX}/X^D$ tra/tra	30	7.4±1.2	44.6±2.7

[a]The number of bristles in genotypes with $\overline{XX}/X^P$ was the same as in the controls $\overline{XX}$/Y.
[b]*sex comb* refers to the basal row of bristles. In males and intersexes, this row is rotated relative to the transversal rows and its bristles are heavier and thicker than those in the transversal rows.
n, number of forelegs.

had a stronger feminizing effect than 2X chromosomes. Contrary to this conclusion, we consider it more plausible that the aneuploid genotype *per se* causes developmental abnormalities which have no relation to sex. In support of our view is the observation that the reduction in sex comb bristles is accompanied by a parallel reduction of all bristles in the transversal rows of the basitarsus (Table I). Structures that were incompletely developed also appeared in $\overline{XX}/X^D$ or $\overline{XX}/X^P$ flies that carried wild-type alleles for all sex-determining genes and were thus pure females. Their vaginal plates were often reduced, spermathecae often missing, and anal plates fragmented.

From our results we conclude that the addition of half an X-chromosome, X^D or X^P, had no feminizing effect on the sexual phenotype of flies that were mutant for any of the autosomal sex-determining genes. When, however, we added these same or even smaller pieces of X material to triploid intersexes (XX; AAA), we observed a strong feminizing effect. The genitalia and analia became entirely female; all flies with X^D and most flies with X^P had seven tergites and sternites which is characteristic of females; the sex combs, a male structure that is always present in XX; AAA flies, completely disappeared when X^D pieces were added, and were only occasionally present (on seven out of 22 forelegs) when X^P pieces were added (data not shown).

As a general conclusion from our experiments we visualize the X:A ratio as the primary genetic signal that implements a specific activity at a small number of regulatory genes (*ix, tra, tra-2, dsx*) acting at a lower hierarchical level. The state of activity of these genes then determines and maintains the sexual pathway.

Here, we must point out an important difference between triploid intersexes and those intersexual phenotypes that are produced by *ix* or *dsx*. The triploid intersexes display a mosaic of purely male and female cells, similar to gynandromorphs (Dobzhansky and Bridges, 1928; Hannah-Alava and Stern, 1957; Stern, 1966), whereas the mutants *ix* or *dsx* represent truly intermediate forms between maleness and femaleness, even at the cellular level (Roost *et al.*, 1979; Baker and Ridge, 1980; Epper, 1981). In triploid intersexes, all sex-determining genes are present in the wild-type form (ix^+, tra^+, dsx^+) and can be normally regulated. To the intermediate X:A ratio of 0.67 (XX; AAA) some cells res-

pond by choosing the female pathway, some cells by choosing the male pathway, by correspondingly setting the state of activity at ix^+, tra^+, dsx^+. This proportion is shifted in favor of more cells turning on the female program when pieces of an X chromosome carrying female determinants are added. The particular X^D that we used in our experiments was sufficient to convince all cells to implement the female program. On the other hand, when one of the autosomal genes *ix, tra* or *dsx* was mutant, the mutant state of this gene determined the sexual pathway, and the addition of more X-chromosome material carrying female determinants, e.g., X^D, remained without effect. This becomes especially clear for the genotype $dsx^D/+$. The addition of X^D or X^P is unable to suppress the masculinizing effect of dsx^D, or to increase the feminizing effect of the wild-type allele that is apparently already fully active with two X chromosomes.

The sex-determining genes have been compared with homeotic genes (Baker and Ridge, 1980; Wieschaus and Nöthiger, 1982). They are homeotic in the sense that their state of activity selects one of two mutually exclusive developmental programs, just as the gene bithorax (*bx*) selects the metathoracic (when active) or the mesothoracic (when inactive) pathway (Lewis, 1978). As an analogy to our experiments, let us consider the gene Polycomb (*Pc*) and the genes of the bithorax complex (BX-C) (Lewis, 1978). Pc^+ is a genetic signal, as is the X:A ratio; it acts to repress the BX-C genes in a segment-specific pattern. The state of activity of the BX-C genes determines the segmental identity, just as ix^+, tra^+, dsx^+ determine the sexual pathway. A deletion for *Pc* results in the complete derepression of the entire BX-C so that all segments are identical and like the last abdominal segment. When some of the BX-C genes are mutant, however, then their mutant state, corresponding to the repressed state, determines the segment identity, even under conditions of complete derepression of the BX-C. Similarly, when a sex-determining gene is mutant, its mutant state determines the sexual phenotype, irrespective of the X:A signal. In regulatory pathways, the general rule is that the mutant state of the gene that is lowest in the hierarchy determines the developmental program.

Materials and methods

Since triplo-X flies (XXX; AA) are usually lethal with rare escapers, we constructed flies that had attached X-chromosomes (XX) plus either a distal half (X^D) or proximal half (X^P) of a third X, and were mutant for one of the autosomal sex determining genes (Figure 1). Such flies (XX+ ½X) are viable, and in the absence of mutations at any of the autosomal sex-determining loci, are weakly fertile females. The crosses described in Figure 1 produced, among others, the two desired genotypes and the control. The analysis consisted in a microscopical examination of sexually dimorphic structures, such as the basitarsus of the foreleg where the males carry a row of heavy thick bristles, the sex comb, and the terminal abdominal segments, especially the genitalia and analia where the sexual differences are most conspicuous. The cultures were grown on standard medium and kept at 25°C.

Acknowledgements

We want to thank Dr. J.R. Merriam for kindly providing the stock with T(X;Y)B26, Ms. A. Kohl for drawing the figures, and Ms. M. Eich for developing the photographs. We appreciate the fruitful discussions with Drs. T. Schüpbach and E. Wieschaus, and we thank Dr. A. Dübendorfer, A. Hilfiker, M. Leuthold and H. Schmid for useful comments on the manuscript. Supported by Swiss National Science Foundation grant 3.237-0.82, by the 'Stiftung für wissenschaftliche Forschung an der Universität Zürich' and by the Julius Klaus-Stiftung.

References

Baker,B.S. and Ridge,K.A. (1980) *Genetics*, **94**, 383-423.
Baker,B.S. and Belote,J.M. (1983) *Annu. Rev. Genet.*, **17**, 345-393.
Bridges,C.B. (1921) *Science (Wash.)*, **54**, 252-254.
Bridges,C.B. (1925) *Am. Nat.*, **59**, 127-137.
Dobzhansky,T. and Bridges,C.B. (1928) *Am. Nat.*, **56**, 261-271.
Dobzhansky,T. and Schultz,J. (1934) *J. Genet.*, **28**, 349-386.
Duncan,I.W. and Kaufman,T.C. (1975) *Genetics*, **80**, 733-752.
Epper,F. (1981) *Dev. Biol.*, **88**, 104-114.
Epper,F. and Nöthiger,R. (1982) *Dev. Biol.*, **94**, 163-175.
Hannah-Alava,A. and Stern,C. (1957) *J. Exp. Zool.*, **134**, 533-556.
Hildreth,P.E. (1965) *Genetics*, **51**, 659-678.
Hodgkin,J. (1983) *Molec. Gen. Genet.*, **192**, 452-458.
Janning,W., Labhart,C. and Nöthiger,R. (1983) *Wilhelm Roux's Arch. Dev. Biol.*, **192**, 337-346.
Lewis,E.B. (1978) *Nature*, **276**, 565-570.
Lindsley,D.L. and Grell,E.H. (1968) *Carnegie Inst., Washington, Publ. No.* **627**.
Morgan,T., Redfield,H. and Morgan,L.V. (1943) *Carnegie Inst.*, Washington, Year Book, **42**.
Nöthiger,R., Dübendorfer,A. and Epper,F. (1977) *Wilhelm Roux's Arch. Dev. Biol.*, **181**, 367-373.
Roost,M., Andersen,N., Keller,W.F.C., Schmid,H. and Nöthiger,R. (1979) *European Drosophila Research Conference, Kupari, Abstr.* **6**.
Sanchez-Herrero,E., Vernos,I., Marco,R. and Morata,G. (1985) *Nature*, in press.
Schüpbach,T., Wieschaus,E. and Nöthiger,R. (1978) *Wilhelm Roux's Arch. Dev. Biol.*, **185**, 249-270.
Stern,C. (1966) *Rev. Suisse Zool.*, **73**, 339-355.
Stewart,B. and Merriam,J.R. (1975) *Genetics*, **79**, 635-647.
Sturtevant,A.H. (1945) *Genetics*, **30**, 297-299.
Watanabe,T.K. (1975) *Jap. J. Genet.*, **50**, 269-271.
Wieschaus,E. and Nöthiger,R. (1982) *Dev. Biol.*, **90**, 320-334.

Received on 31 August 1984; revised on 29 October 1984