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Sex determination and dosage compensation in *Drosophila melanogaster*: production of male clones in XX females

L.Sánchez[1] and R.Nöthiger*

Zoological Institute, University of Zürich-Irchel, CH-8057 Zürich, Switzerland

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Sex determination and dosage compensation in *Drosophila* are implemented by the ratio of X-chromosomes to sets of autosomes (X:A ratio). Our aim was to change this X:A ratio during development, and to assess the response of the affected cells in sexually dimorphic structures. For this purpose, clones of XO constitution were produced in female embryos and larvae of two genotypes in which almost the entire euchromatic arm of one X-chromosome was translocated to the third chromosome. Genotype I was heterozygous for the X-linked recessive mutations Sxl^{fLS}, genotype II was homozygous for Sxl^{+}. The Sxl^{+} gene (sex-lethal) is involved in mediating sex determination and dosage compensation. In genotype I (Sxl^{fLS}), male clones could be generated up to 48 h in genitalia and analia, up to 72 h in the sex comb region and up to 96 h in 5th and 6th tergites. In genotype II (Sxl^{+}), male clones only appeared in the tergites, and only up to 24 h. The difference in these results is ascribed to the presence of Sxl^{fLS} in genotype I: when homozygous, this mutation causes XX clones to differentiate male structures; most of the male clones produced in genotype I must therefore be XX. In contrast, male clones produced in genotype II must be XO. Since these were only found when generated in embryos we conclude that the X:A ratio expresses itself autonomously in clones by setting the state of activity of the *Sxl* gene around blastoderm stage. Once this is achieved, the X:A signal is no longer needed, and the state of activity of the Sxl^{+} gene determines sex and dosage compensation.

Key words: clonal analysis/dosage compensation/*Drosophila*/sex determination/X-chromosomes

Introduction

In *Drosophila*, sex determination and dosage compensation (for review, see Stewart and Merriam, 1980) are intimately related, and both are dependent on the ratio of X-chromosomes to sets of autosomes (X:A ratio). Based on genotypes with variable X:A ratios, Bridges (1921, 1925) formulated his classic 'balance concept' of sex determination. This hypothesis visualizes sex as a quantitative character with continuous variation. Female-determining genes are assumed to be located on the X-chromosome, male-determining genes on the autosomes. The balance between these two opposing forces determines the sex of the zygote, whereby 2X outweigh 2A, but 2A outweigh 1X.

An important insight into the mechanisms of sex determination and differentiation was gained from studies with gynandromorphs. These mosaic flies demonstrate that the X:A ratio expresses itself autonomously in individual cells. Even when a mosaic female contains only a few X;2A cells as a small patch in a sexually dimorphic region, the cells of this island differentiate typical male structures (Stern and Hannah, 1950; Kroeger, 1959). In gynandromorphs, the loss of an X-chromosome occurs during early nuclear cleavage long before cells are formed (Zalokar *et al.*, 1980). Illmensee (1973, 1978) has shown that the cleavage nuclei of *Drosophila* are totipotent whereas the fate of blastoderm cells is determined, probably for segment-specific development (Wieschaus and Gehring, 1976; Steiner, 1976). It is therefore conceivable that the sexual pathway and dosage compensation, too, may become determined once cells have formed in the blastoderm stage. The clonal descendants of a cell that had lost one of its X-chromosomes at, or some time after, the blastoderm stage, might thus remain in the female pathway. If the rate of transcription of the single X-chromosome allowed development of the clone, female structures would be differentiated despite the male X:A ratio of these cells. These considerations led us to ask the following questions. (1) Can a cell whose genotype is changed from XX to XO after the blastoderm stage adjust its dosage compensation to the new X:A ratio? (2) What sexual phenotype do such clones show? If they enter the male pathway, up to what time in development is 'sex reversal' possible?

We have constructed a genotype that allowed us to remove, by mitotic recombination induced by X-irradiation, one of the X-chromosomes from a cell at different times after blastoderm formation, and thus to produce X;2A clones in XX;2A female flies.

In the course of our experiments we discovered a mutation located on the X-bearing element of the reciprocal translocation *T(1;3)OR 17* (see Figure 1), that we identified as an allele of Sxl^{f} (sex-lethal female-specific), a gene required for female sexual development (Cline, 1979; Sánchez and Nöthiger, 1982), and probably also for proper adjustment of dosage compensation (Cline, 1978; Lucchesi and Skripsky, 1981). The presence of this mutation, which we named Sxl^{fLS}, obscured the origin of the male clones that appeared in our experiments. These clones could consist either of X;2A cells, or of 2X;2A cells homozygous for Sxl^{fLS} (Figure 1). As shown previously (Cline, 1979; Sánchez and Nöthiger, 1982), clones homozygous for Sxl^{f} or Sxl^{fLS} differentiate male structures. After we discovered Sxl^{fLS} in our translocation *T(1;3)OR 17*, we removed the mutation and repeated the experiment. This provided an opportunity for studying XO clones with and without the Sxl^{f} mutation, and to propose a hypothesis about when and how the X:A ratio and the Sxl^{+} gene act to implement the sexual pathway (Figure 4).

Results

Clones in the external derivatives of the genital disc

Quantitative analysis. Table I presents the data on frequencies of the different male clones that we obtained, and Figure 3a, b shows two examples.

Genotype I (Sxl). Following mitotic recombination induced by X-irradiation we found male clones in the genitalia

[1]Present address: European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg 1, FRG.

*To whom reprint requests should be sent.

and analia. In striking contrast to yellow female clones in the analia (Figure 2a), the frequency of male clones decreased with age at irradiation and dropped drastically after 48 h (Table I). No male genital clones and only two male anal clones, each consisting of a single bristle, were found after 48 h. These two anal clones represent cases in which the small size of the bristles and their position, protruding from the female anal plate, identified them as male.

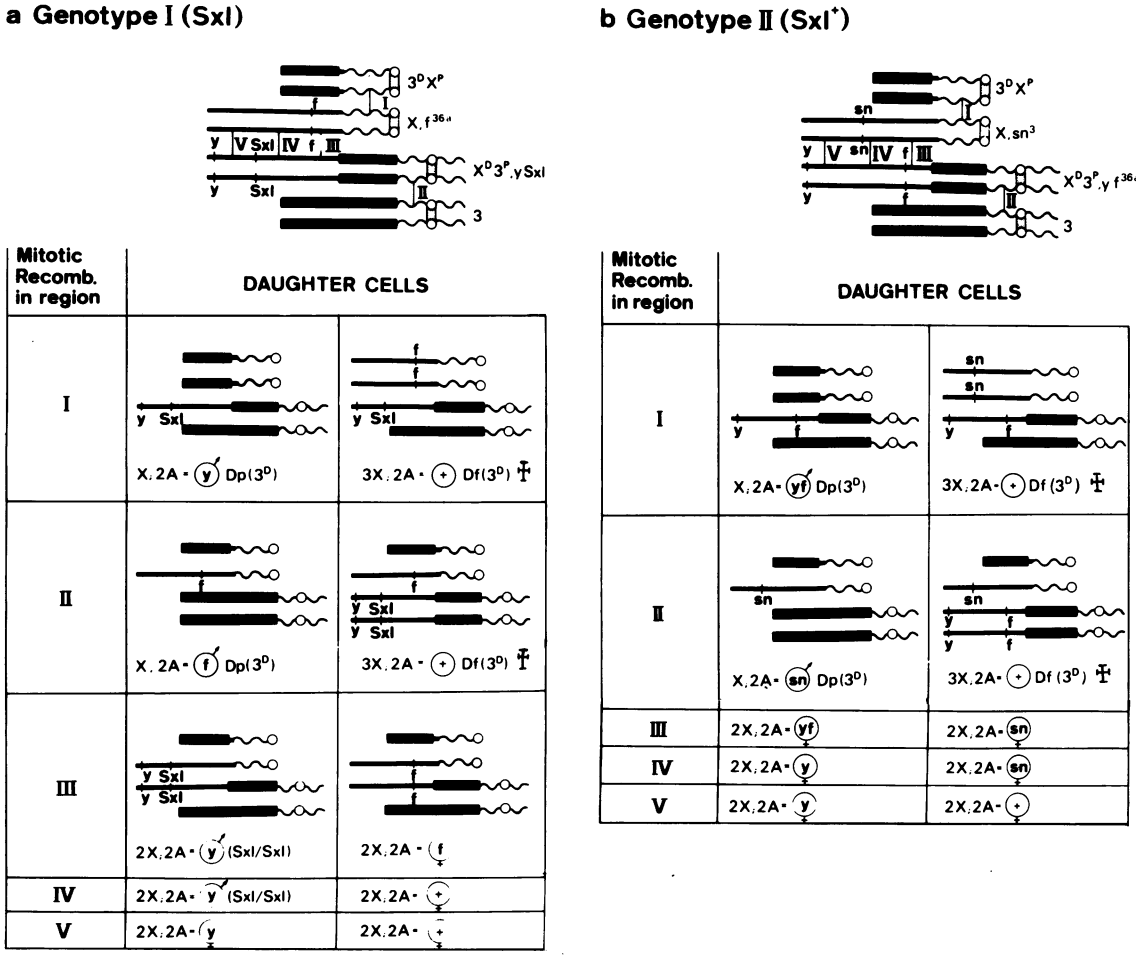

Fig. 1. Schematic representation of genotypes, and consequences in daughter cells after mitotic recombination in different chromosomal regions (I – V). Thin bar, X-chromosome; heavy bar, left arm of chromosome 3; wavy line, heterochromatin. The two elements of the translocation *T(1;3)OR 17* are designated by $3^{D}X^{P}$ and $X^{D}3^{P}$. *Sxl*, Sxl^{fLS}; *y*, *f*, *sn*, cell markers *yellow (y)*, *forked* (f^{36a}), *singed* (sn^{3}); †, the 3X;2A clones are probably inviable due to hyperploidy for an entire X and hypoploidy for 3^{D}. (**a**) Genotype heterozygous for Sxl^{fLS}, designated as genotype I *(Sxl)*; (**b**) genotype with Sxl^{+}, designated as genotype II (Sxl^{+}).

Table I. Number and frequency (%) of male clones in female genital discs

Age at irradiation (h)	[a]Genotype I (Sxl^{fLS})					[a]Genotype II (Sxl^{+})			
	Number of flies	In genitalia	In analia			Number of flies	In genitalia	In analia	
			y	*f*	♂*y*//*f*♀ twins			*y f*	*sn*
3	709	13 (1.8%)	12 (1.7%)	0	1 (y)	391	0	0	0
24	1061	12 (1.1%)	8 (0.7%)	0	0	498	0	0	0
48	738	5 (0.7%)	3 (0.4%)	0	0	190	0	0	0
72	772	0	1	0	0	138	0	0	0
96	358	0	1	0	0	165	0	0	0
Unirradiated control	398	0	0	0	0	105	0	0	0

[a]For complete genotype see Figure 1.

Since the size of male genital clones is difficult to quantify, data on clone sizes are only given for the claspers (Table II). The apparent decrease in clone size between 3 and 24 h is not significant. The size of the male clones in the analia decreased significantly between 3 and 24 h.

All male clones were marked with *yellow*, never with *forked*. With the only exception of one *y* male anal clone generated at blastoderm stage, we found no *yellow//forked* twin clones exhibiting a male phenotype in the *yellow* partner. The frequency of female *forked* clones (Figure 2a) was very low compared with the frequency of female *yellow* clones, and reached an appreciable frequency only at 96 h.

Genotype II (Sxl$^+$). We did not find any male clone in the genitalia or analia (Table I). The frequency of female *yellow* and *yellow-forked* clones in the analia (Figure 2a) increased during development as expected. Due to the poor expressivity of sn^3 in the analia, *singed* clones were not considered.

Qualitative aspects. In most cases (21/25), the presence of male genital elements was accompanied by a complete set of female genitalia (Figure 3a). The four exceptional cases were small parts of the hypandrium which were associated with deficiencies in the vaginal plates (VP) and in the 8th tergite (T8). Male clones in the anal plates were correlated with the absence of a corresponding female part, and two of the 24 anal clones were accompanied by elements of the penis apparatus (dorsal paramere in Figure 3b). These results are identical to those obtained by Wieschaus and Nöthiger (1982) and confirm their conclusions about the cell lineage relationships between the derivatives of the genital disc.

In all cases there was a complete correspondence between the mutant marker and the male phenotype. This shows that the clones of male structures resulted from mitotic recombination, and that the expression of their genotypic sex is autonomous.

Clones in the forelegs

The data in Table III show that sex comb bristles, a typical male character, were obtained in female foreleg basitarsi with constant frequency up to 72 h, but not later, and only in flies of genotype I *(Sxl)*. With the exception of two blastoderm clones which produced *forked* sex combs, the sex comb bristles were always *yellow*. Twice at 24 h and twice at 48 h *yellow* sex combs appeared in twin with female *forked* bristles. The size of the sex comb clones dropped after blastoderm and remained constant for later stages (Table II). Figure 2b shows the frequency of *yellow* clones in the foreleg segments coxa, trochanter, femur and tibia; the transversal rows were excluded since *y* is an unreliable marker for these bristles. In both genotypes, the frequency of *yellow* clones increased during development as expected. The absence of sex comb clones at blastoderm stage in genotype II (*Sxl*$^+$) is not significant ($p < 0.05$), but the sum of 26 clones in genotype I, and zero in genotype II represents a significant difference.

The sex comb bristles exhibited normal morphology, and in some cases (Figure 3c) even were rotated (Tokunaga and Stern, 1965), although this rotation was usually incomplete. As a rule, the sex comb bristles replaced the corresponding female bristles in the region of the 7th and 8th transversal rows of the basitarsus, as already reported by Tokunaga (1962) for sexual mosaics.

Clones in the 5th and 6th tergites

Table IV shows the frequencies of male clones in the unpigmented area of the 5th and 6th tergites where the sex of clones can be assessed. The table also contains the data for female clones. With genotype I *(Sxl)* we obtained *yellow* and *forked* male clones at blastoderm stage, but at later stages only *yellow* male clones appeared. At all stages, we found *yellow//forked* twin clones associated with male pigmentation in the *yellow* partner. We also obtained female clones showing the following phenotypes: *yellow, forked* and *yellow//forked* twins. The origin of these female twin clones is obscure. With genotype II (*Sxl*$^+$) we found *yellow-forked* and *singed* male clones at blastoderm stage, but except for three clones at 24 h, no more male clones appeared at later

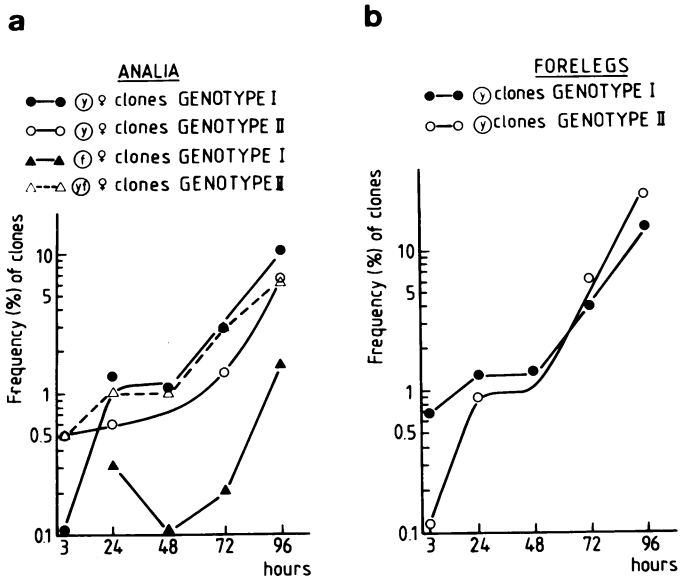

Fig. 2. Frequency of clones (ordinate) as a function of age at irradiation (abscissa). For genetic constitution, phenotypes and origin of clones see Figure 1. **(a)** Female clones in anal plates; **(b)** clones in forelegs (coxa, trochanter, femur, tibia).

Table II. Size of male clones (average number of bristles per clone ± standard error)

Age at irradiation (h)	[a]Genotype I (*Sxl^{fLS}*)						[a]Genotype II (*Sxl*$^+$)	
	Clasper	Analia	5th + 6th tergites		Sex combs		5th + 6th tergites	
			y	*f*	*y*	*f*	*y f*	*sn*
3	13.0 ± 2.8	22.5 ± 3.1	11.2 ± 1.7	12.1 ± 2.3	4.5 ± 1.1	4.5	8.8 ± 2.7	10.4 ± 1.7
24	9.0 ± 3.4	6.3 ± 1.1	3.8 ± 0.4		1.1 ± 0.3		3.6 ± 0.8	
48		5.0	3.1 ± 0.3		1.6 ± 0.4			
72		1	3.4 ± 0.5		1			
96		1	2.0					

[a]For complete genotype see Figure 1.

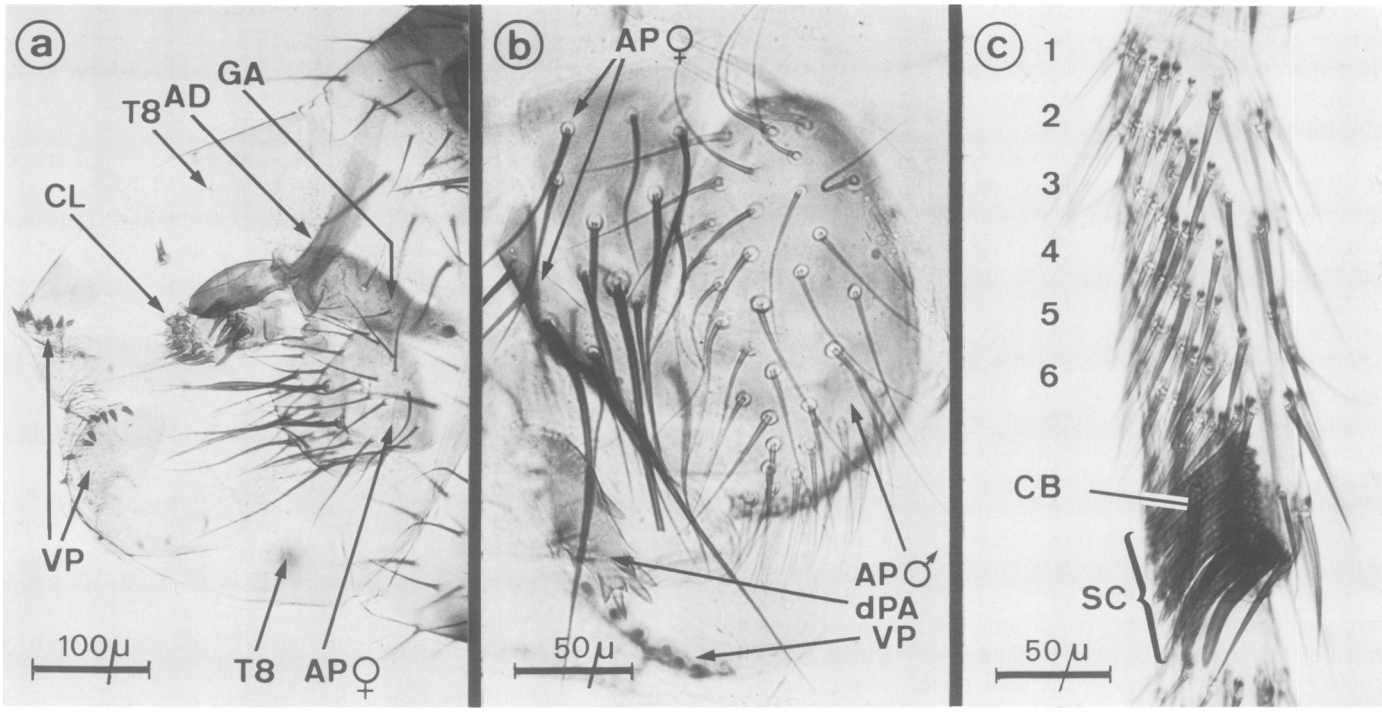

Fig. 3. Photographs of *y* male clones induced at 3 h in genotype *(Sxl)*. **(a)** Clone of genital arch *(GA)*, apodeme *(AD)*, and clasper *(CL)* branching off from a female tergite 8 *(T8)*. The female terminalia are complete: *AP*, ♀ anal plates; *VP*, vaginal plates. **(b)** Clone of a male anal plate *(AP ♂)* and a dorsal paramere *(dPA)*. The female genitalia were complete (only one vaginal plate *(VP)* is shown), but of the anal plates only the left halves were present *(AP ♀)*. **(c)** Basitarsus of foreleg with clone of sex comb *(SC)* and central bristle *(CB)*; 1–6, transversal rows.

Table III. Numbers and frequencies (%) of male clones in female foreleg basitarsi

Age at irradiation (h)	[a]Genotype I (Sxl^{fLS})				[a]Genotype II (Sxl^{+})		
	Number of forelegs	Sex comb bristles			Number of forelegs	Sex comb bristles	
		y	*f*	♂*y*//*f*♀		*y f*	*sn*
3	1438	6 (0.4%)	2 (0.1%)	0	796	0	0
24	2076	6 (0.3%)	0	2 (0.1%)	1100	0	0
48	1253	4 (0.3%)	0	2 (0.15%)	–	–	–
72	1511	4 (0.3%)	0	0	544	0	0
96	1444	0	0	0	148	0	0
Unirradiated control	895	0	0	0	439	0	0

[a]For complete genotype see Figure 1.

stages, when female clones were still produced. The size of the male clones at blastoderm stage is similar in genotypes I and II (Table II).

Discussion

The origin of male clones

The most conspicuous, and for our interpretation (see Figure 4) most important, observation is the difference in the results obtained with genotype I *(Sxl)* and genotype II (Sxl^{+}). With genotype I (see Figure 1a), male clones could be generated up to 48 h or even later, and in all sexually dimorphic regions. With genotype II (see Figure 1b), male clones only appeared when irradiation was carried out at 3 h and 24 h, and only in the tergites. How are we to interpret these differences?

We think that they can be ascribed to the presence of Sxl^{fLS}, an allele of *Fl* (Muller and Zimmering, 1960), in genotype I, whereas genotype II carries the wild-type allele of this gene. The work of Cline (1978, 1979) and our own work (Sánchez and Nöthiger, 1982) had shown that Sxl^{+} is required to maintain the female developmental program in 2X;2A larvae: when mitotic recombination rendered a cell homozygous for Sxl^{f} in a heterozygous $Sxl^{f}/+$ larva, the clonal descendants of this cell differentiated male structures. To explain the sex-specific lethality of mutations at the *Sxl* locus, Cline (1978) furthermore suggested that this locus is involved in dosage compensation. This process adjusts the rate of transcription of X-chromosomal genes in males and females such that the single X-chromosome in X;2A animals produces as many transcripts as the two X-chromosomes in 2X;2A animals. The product of Sxl^{+} is thus necessary in females to install a low rate of transcription and to maintain the developmental system in the female pathway. This hypothesis has recently received some support from studies which measured a higher transcriptional activity in XX larvae mutant for Sxl^{f} than in normal females (Lucchesi and Skripsky, 1981).

Our results with genotype II in which no male clones could be produced during larval stages suggest that dosage compensation and sex determination become irreversibly adjusted at or shortly after blastoderm stage. Whereas XO clones that are generated in XX embryos during nuclear cleavage develop normally and produce male structures giving rise to gynandromorphs, our attempts to generate such clones by mitotic recombination after cellularisation largely failed. The

Table IV. Clones in 5th and 6th tergites

Age at irradiation (h)	[a]Genotype I (*Sxl*^{fLS})							[a]Genotype II (*Sxl*$^{+}$)						
	Number of abdomens	Male clones			Females clones			Number of abdomens	Male clones		Female clones			
		y	*f*	♂*y*//*f*♀ twins	*y*	*f*	*y*//*f*^{e} twins		*y f*	*sn*	*y f*	*sn*	*y*	*y f*//*sn* twins
3	709	22[b]	13	2	21	25	10	397	8	17	8	9	7	41
		(19)[c]	(11)	(0)	(18)	(22)				(10)	(0)	(5)		(26)
		2.6[d]	1.5%		2.5%	3.1%	1.4%		2.1%	2.5%		1.2%	1.7%	6.5%
24	1061	28	1	27	92	34	4	489	3	3	26	12	13	61
		(23)	(0)	(16)	(87)	(29)				(0)	(0)	(8)		(43)
		2.1%		1.5%	8.1%	2.7%	0.3%		0.6%			1.6%	2.6%	8.7%
48	738	19	2	24	74	24	16		0	1	15	7	22	23
		(15)	(0)	(17)	(70)	(20)		190		(0)	(0)	(5)		(16)
		2.0%		2.3%	9.4%	2.7%	2.1%					2.6%	11.5%	8.4%
72	772	11	2	24	37	16	8	138	0	1	5	5	10	8
		(7)	(0)	(16)	(33)	(12)				(0)	(0)	(4)		(3)
		0.9%		2.0%	4.2%	1.5%	1.0%					2.9%	7.2%	2.1%
96	358	4	2	8	22	7	5	164	0	3	8	0	6	11
		(2)	(1)	(4)	(20)	(5)				(0)	(0)			(5)
		0.5%		1.1%	5.5%	1.3%	1.3%						3.6%	3.1%
Unirradiated control	398	2	1	4	2	2	0	109	0	2	9	1	0	4

[a]For complete genotype see Figure 1.
[b]Observed number of clones.
[c]Corrected number of clones after subtraction of control values.
[d]Frequencies (%); calculated from corrected number of clones.
[e]The origin of these clones is obscure unless one postulates multiple recombinational events.

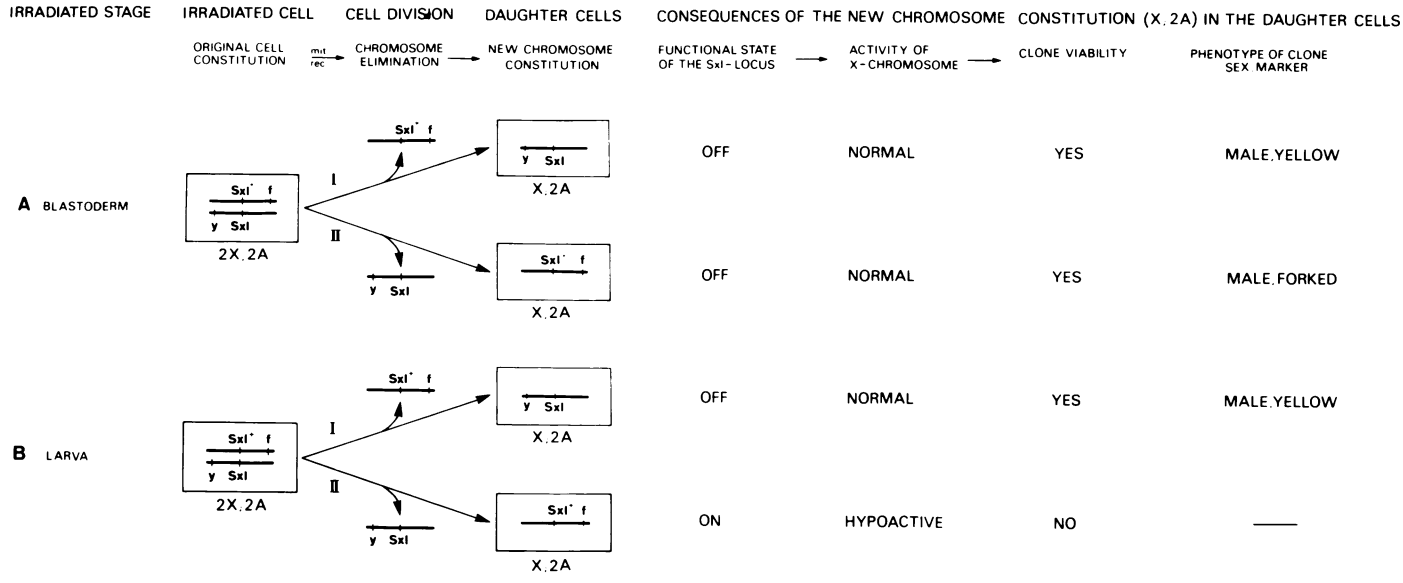

Fig. 4. Fate of XO clones (X;2A) generated in genotype I *(Sxl)*, illustrating how we visualize the action of the X:A ratio and the role of the *Sxl* gene in sex determination and dosage compensation. Mitotic recombinatin (mit. rec.) in region I or II (see Figure 1) yields X;2A cells (clones) that are marked with either *y* or *f*, and carry either the mutant *Sxl*^{fLS} or the wild-type *Sxl*$^{+}$ allele. One X-chromosome is removed either before (A, blastoderm) or after (B, larva) the signal of the X:A ratio has implemented the sexual pathway by setting the state of activity of the *Sxl* gene (see Discussion).

resulting XO genotype either acts as a cell lethal, or more likely the clones succumb to cell competition (Morata and Ripoll, 1975; Simpson, 1979) because the single X-chromosome is transcribed at the low female rate which makes the clone grow more slowly than the surrounding normal female cells, similar to *Minute* clones generated in a wild-type larva (Morata and Ripoll, 1975). (As described in Materials and methods, trisomy for 3^{D} allows normal development of clones and is therefore not the cause for the slow growth.)

The male clones generated in genotype I *(Sxl)* must then mainly be due to homozygosity for *Sxl*^{fLS}, and thus have a 2X;2A constitution. We know that such clones can be produced in heterozygous females in which they form male structures (Cline, 1979; Sanchez and Nöthiger, 1982). Their yellow phenotype is a strong indication of this although mitotic recombination in region I could also produce yellow clones with XO constitution (Figure 1a). Some of the *yellow* male clones could in fact be XO if in females the wild-type product

of Sxl^+ were continuously required to keep the transcriptional activity low. After removal of Sxl^+, the *yellow* XO cell is also mutant for Sxl^{fLS}. This could lead to a high male rate of transcription which would save the XO clone and direct it into the male pathway. Our experiments did not show whether or not this is the case, but we return to this issue below.

We have summarized our view in Figure 4. In conclusion, we visualize the X:A ratio as a primary signal that regulates the activity of Sxl^+ at or shortly after the blastoderm stage such that this gene is active when the X:A ratio equals 1.0, as in normal females, and inactive when it equals 0.5, as in normal males. Once this is achieved, the X:A signal is no longer needed, and the state of activity at the *Sxl* locus remains set. Even when a X-chromosome is removed, the Sxl^+ product continues to be made which results in a fatal deficit of X-chromosomal gene products in such XO clones. If, however, the single X of the XO clone is mutant for Sxl^{fLS}, its rate of transcription may become high as in normal males allowing XO clones to survive and to form male structures.

Time- and tissue-specificity

Two observations merit discussion, namely: (i) that in genotype II, viable male X;2A clones could only be generated in tergites and only up to 24 h (Table IV), whereas male clones homozygous for Sxl^{fLS} (genotype I) appeared in all regions studied and after much later irradiation times; and (ii) that homozygous Sxl^{fLS} clones could be generated up to 48 h in the genital disc, up to 72 h in the sex comb region, and throughout the larval period in the tergites (Tables I, III, IV).

The precursor cells for the tergites, the so-called histoblasts, undergo one division during embryogenesis, but then do not divide until puparium formation when a series of rapid cell divisions takes place (Garcia-Bellido and Merriam, 1971a; Guerra *et al.*, 1973; Madhavan and Scheiderman, 1977; Szabad *et al.*, 1979). The histoblasts are special in some other respects. Cell competition, which is very effective in imaginal discs, is practically absent among histoblasts (no *Minute* effect: Morata and Ripoll, 1975; Ripoll, 1977; Simpson, 1979, 1981), and furthermore, homozygous clones of many lethal mutations that are inviable in the thoracic discs, form normal structures in the tergites (Ripoll, 1977). It is, therefore, possible that the X;2A clones survive in the tergites although their single X is only transcribed at the low rate typical for females. This means that dosage compensation and sex determination could have taken place around the blastoderm stage in all cells, but development of XO clones that grow slowly because their single X is hypoactive, is impeded to different degrees in different cell types, depending on the dynamics of cell growth, and is almost normal in tergites.

If sex and dosage compensation are implemented around the blastoderm stage, X;2A clones should follow the female pathway. In the foreleg and genital discs, such clones will be competed out, whereas they may survive in the tergites and form a female pattern. We might expect that these XO clones are smaller than normal XX clones. However, when we measured clone sizes, all female clones were of about the same size.

An argument in support of our speculations may be derived from Figure 2a which shows that *forked* female clones in the analia are very rare for early irradiation times, but reach a high frequency at 96 h. These clones could be the partners of y♂//f♀ twins generated by mitotic recombination in region III (Figure 1a), but such twin clones are extremely rare in the genital disc (one case at 3 h, Table I). Since region III is a very small chromosomal interval, we consider it more likely that the *forked* clones arose by mitotic recombination in the larger region II and are thus XO. When generated early in development, such clones are competed out in the analia whereas clones generated later may survive, but then maintain the female developmental program. The phenomenon is again reminiscent of *Minute* clones that can only be found when produced late in development (Morata and Ripoll, 1975).

In contrast to X;2A clones, XX clones homozygous for Sxl^f develop almost normally although the mutant state of Sxl^f should lead to a high (male) level of X-chromosome transcription. If this were the case, the resulting excess of X-chromosomal gene products is apparently much better tolerated than the corresponding deficit just as trisomies are better tolerated than monosomies (Stewart and Merriam, 1973).

The observation that male clones can be generated up to different stages in different imaginal cells was also made by Wieschaus and Nöthiger (1982) in their clonal analysis of *tra-2* and *tra*, two mutations that transform females into males. The phenomenon is best explained by assuming that the products of Sxl^+ are passed on to the daughter cells and still exert their effects after the wild-type allele has been removed from a cell (perdurance effect: Garcia-Bellido and Merriam, 1971b). Depending on the specific growth dynamics of a primordium, the minimum number of cell divisions needed to abolish the effects of the Sxl^+ products will require different periods of time. The reader will find a detailed discussion in the paper by Wieschaus and Nöthiger (1982).

Materials and methods

The genetic system

The genetic system that we employed to remove an X-chromosome from a cell in a developing female makes use of a reciprocal translocation, *T(1;3)OR 17*, in which almost the entire euchromatic arm of the X-chromosome is translocated on to the left arm of chromosome 3 (breakpoints 19E; 67C). For a description of mutations and chromosomes see Lindsley and Grell (1968). The genotypes of the two kinds of females that were irradiated to induce mitotic recombination are presented in Figure 1. A description of the different types of expected clones follows.

Genotype I (Sxl = Sxl^{fLS}, Sanchez and Nöthiger, 1982) (Figure 1a). Depending on the region of the exchange and on the chromosomes involved, appropriate segregation should lead to X;2A cells that are either marked with *y* (region I) or with f^{36a} (region II). Mitotic recombination in region III or IV gives rise to *yellow* clones that represent 2X;2A cells homozygous for Sxl^{fLS}. These clones can be in twin with *forked* female clones when mitotic recombination took place in region III. Mitotic recombination in region V gives rise to *yellow* (Sxl^+) female clones.

Genotype II (Sxl^+) (Figure 1b). Mitotic recombination in region I and II gives rise to X;2A clones marked with *y* f^{36a} (region I) or sn^3 (region II). Mitotic recombination in regions III, IV and V generates female clones marked with *y* f^{36a} in twin with sn^3 (region III), *y* in twin with sn^3 (region IV) and *y* (region V).

In both genotypes, the X;2A clones are hyperploid for the distal arm of chromosome 3 (3^D) which should, however, not affect cell viability since this duplication is compatible with survival of male flies (Lindsley and Grell, 1968; Sánchez, unpublished data), and duplications of this size are also viable in clones (Ripoll, 1980). The 3X;2A clones are not marked and probably inviable (see legend to Figure 1).

Identification of the sex of clones

The sexual phenotype of the marked clones was assessed in sexually dimorphic regions of the fly (sex comb region on basitarsus of foreleg, 5th and 6th tergites, external derivatives of the genital disc). For a description of the morphology of these structures see Bryant (1978). Male clones in a female foreleg basitarsus were defined by the presence of sex comb bristles. In the tergites, the male clones were defined by the presence of marked bristles associated with male pigmentation in the unpigmented area of the female 5th and 6th tergites. In the external derivatives of the genital disc, male genital elements, as

well as larger male clones in the anal plates, can easily be recognized. Small anal clones, however, could only be identified as male when they comprised the ventral part of a male anal plate where size and density of bristles clearly differ from the female pattern. Marked clones whose sex was doubtful were excluded from the analysis.

Mitotic recombination

Mitotic recombination was induced by 1000 rad X-rays applied at different times of development. Blastoderm stages of genotype I were irradiated with a Siemens-Dermopan-2 (50 kV, 25 mA, 1.0 mm Al filter, focus distance 30 cm, 213 rad/min). Later stages of genotype I were irradiated with a Picker X-ray machine (200 kV, 12 mA, 1.0 mm Al filter, focus distance 30 cm, 227 rad/min). All stages of genotype II were irradiated with a Philips X-ray machine (150 kV, 14 mA, 2.0 mm Al filter, 1.0 mm plexiglass filter, focus distance 25 cm, 500 rad/min). Embryos and larval stages of 24 h and 48 h were irradiated on the food; larvae of later stages (72 h and 96 h) were washed out of the food and wrapped in a fine nylon cloth for irradiation.

Culture and preparation of flies

Flies were raised on standard food at 25°C. The age of the animals is given in hours after oviposition. For inspection under a compound microscope, the flies were macerated in hot 10% KOH for 6 min, thoroughly washed with H_2O, and mounted in Faure's solution.

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