THE ROLE OF DOSAGE OF THE REGION 7D1–7D5-6 OF THE *X* CHROMOSOME IN THE PRODUCTION OF HOMEOTIC TRANSFORMATIONS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

A high frequency of homeotic transformations appears in *Df(3)red*/+ progeny of *Df(1)sn*^{C128} /+ females. Generally, the metathoracic appendages are partially transformed into mesothoracic ones. *Df(1)sn*^{C128} includes a small region of the *X* chromosome: 7D1 to 7D5-6. Hypodosage of this region is mainly effective at the level of the maternal genotype, and the effect is probably due to hypodosage of the wild-type allele of the gene *fs(1)h*. *Df(3)red* has an effect that is mainly, if not exclusively, zygotic, probably due to hypodosage of the wild-type allele of *Rg-bx*. The frequencies of transformed flies resulting from the interaction between *Df(1)sn*^{C128} and *Df(3)red* are not very sensitive to external conditions and genetic background. Studies of the interactions between *Df(1)sn*^{C128} and other mutations or deficiencies of *chromosome 3* [*Rg-pbx*, *bx*, *pbx*, *Ubx*^{1}, *Ubx*^{130}, *Ubx*^{80}, *Df(3)P9*] reveal an analogy between the hypodosage effect of region 7D1–7D5-6 and the effects of ether treatment of blastoderm stage eggs. The role of the gene *fs(1)h* in the process of segment determination is discussed in the light of these results.

THERE are a number of reasons for thinking that, in Drosophila, determination—that is, the engagement of cells in a specific pathway of development—takes place in successive stages (see reviews by GARCIA-BELLIDO and CAPDEVILA 1978; GEHRING 1976; MORATA and LAWRENCE 1977). After the segregation of the somatic and germ cell lines, the first stage is probably the determination of segments. Various experiments including micro-cautery at the blastoderm stage (BOWNES and SANG 1974), UV microbeam irradiation (LOHS-SHARDIN *et al.* 1979), cell transplantation (CHAN and GEHRING 1971; ILLMENSEE and MAHOWALD 1974; ILLMENSEE 1976), and clonal analysis (WIESCHAUS and GEHRING 1976; STEINER 1976; LAWRENCE and MORATA 1977, 1979) provide a number of arguments suggesting that segment determination takes place at or shortly after the blastoderm stage. At this stage, the zygotic genome has just become active (ZALOKAR 1976; LAMB and LAIRD 1976; ANDERSON and LENGYEL 1979), and joint participation of the maternal genes and those of the zygote can be envisaged in this important stage of development.

According to recent views, segment determination probably involves two components: (1) Positional information present in the cortex of the egg and

generally considered to be in the form of a gradient of unknown nature (WOLPERT 1969; NÜSSLEIN-VOLHARD 1979b), and (2) Reception and interpretation of positional signals leading to the activation of genes ("Selector" genes according to the nomenclature proposed by GARCIA-BELLIDO 1975), the products of which are necessary continuously throughout development and control, directly or indirectly, the morphological and physiological characteristics of each segment.

The establishment of positional information is probably governed by genes active during oogenesis. Maternal-effect mutations, such as bicaudal and dorsal, (NÜSSLEIN-VOLHARD 1977, 1979a; NÜSSLEIN-VOLHARD *et al.* 1980) present characteristics expected of mutations in genes implicated in this process; bicaudal would be involved in the establishment of an antero-posterior gradient and dorsal in a dorso-ventral gradient.

The complex bithorax region, the anatomy and functioning of which has been extensively studied by LEWIS (1954, 1955, 1963, 1964, 1967, 1978), is composed of a battery of structural genes such as *bx*, *pbx* and *bxd*, the characteristics of which correspond well to those expected of selector genes.

Other genes on chromosome *3* also participate in segment determination: Regulator of postbithorax, *Rg-pbx* (LEWIS 1968) and Regulator of bithorax, *Rg-bx*, the first mutation of which was found by E. B. LEWIS (others have recently been isolated by P. INGHAM 1980 and INGHAM and WHITTLE 1980) and Polycomb, *Pc* (P. H. LEWIS 1947; PURO and NYGREN 1975; LEWIS 1978). In recently proposed models (CAPDEVILA 1977; GARCIA-BELLIDO and CAPDEVILA 1978; LEWIS 1978), it is supposed that these genes play a role in the reception of the positional signals and the activation of the structural genes of the bithorax complex *via* the regulatory zones of the latter. The fact that *Rg-pbx* shows a long perdurance as revealed by the analysis of morphogenetic mosaics, provides an indication that the functional state of the gene is limited to an early stage of embryogenesis (CAPDEVILA 1977). A further argument is based on the effect of mutations or dosage of these genes on the frequency of ether-induced (CAPDEVILA 1977; CAPDEVILA and GARCIA-BELLIDO 1978) or heat-shock-induced (SANTAMARIA 1979) phenocopies of bithorax complex mutations induced at the blastoderm stage.

The purpose of the present article is to reveal an interaction between hypodosage of the region of the *X* chromosome corresponding to deficiency $Df(1)sn^{c128}$ (7D1–7D5-6) and hypodosage or mutations of certain chromosome *3* genes just described, in particular *Rg-bx*. The effect, mainly maternal, of hypoploidy of the region 7D1–7D5-6 of the *X* chromosome suggests the existence in this region of a gene implicated in the establishment of positional information. It is highly probable that a mutation in this gene led to the female sterile, heat-sensitive mutant *fs(1)h*, isolated in our laboratory and previously symbolized by the number *1456* (GANS, AUDIT and MASSON 1975). Indeed, under certain conditions (semi-permissive temperature, hemizygous females), this mutation governs, maternally, the appearance of homeotic transformations of a bithorax type, as well as an absence of metathoracic structures. The properties of *fs(1)h* will be described in another paper (FORQUIGNON, in preparation).

MATERIALS AND METHODS

Description of stocks used: *Df(1)sn^{C128}*, a very short X-ray-induced deficiency extending from 7D1 to 7D5-6, inclusive (LEFEVRE, personal communication), was obtained from G. LEFEVRE. From a single *X* chromosome bearing the deficiency, a *Df(1)sn^{C128}/M5* stock was constructed, which was used in the majority of the experiments described here. *Df(1)sn^{C128}* was the only *sn* deficiency used and will hereafter be abbreviated simply as *Df(1)sn*.

Dp(1;2)FN107 is a duplication of a region of the *X* chromosome extending from 7A8± to 8A5± inserted in a *2LR* inversion with breaks at 58E-F and 32B-C (LEFEVRE, pers. com.). This duplication covers *Df(1)sn^{C128}*. The stock *Df(1)RA2/C(1)DX, y f*; *Dp(1;2)FN107/bw^{D}* was obtained from LEFEVRE; from this, *Df(1)sn^{C128}/C(1)DX, y f*; *Dp(1;2)FN107/bw^{D}* was constructed. *Df(1)RA2* is a deficiency extending from about 7D10 to 8A5 (LEFEVRE, pers. com.).

Df(3)red: *Df(3)red^{P93}, l(3)tr Sb/In(3L)P + In(3R)P18, Me Ubx e^{4}*; *Df(3)red^{1}/TM1* and *Df(3)red^{31}/MRS, Sb* were obtained from A. GARCIA-BELLIDO; *T(2;3)ap^{Xa}Df(3)red^{P1}/TM1* and *T(2;3)ap^{Xa}Df(3)red^{P6}/TM1* were obtained from E. B. LEWIS. We do not know the cytological extent of these deficiencies.

Su-Hw^{2} bx bxd/TM1; *In(3LR)Ubx^{130} ("TM2")/Sb*; *In(3R) Ubx^{80} mwh jv/TM1*; *Df(3) P9/Dp(3)P5*; *bx pbx/TM1*; *ru Rg-pbx e^{s} ca/TM1* and *l(3)Rg-bx sr e^{s}/TM1, ca* were obtained from A. GARCIA-BELLIDO.

For a description of mutants and chromosomal rearrangements, see LINDSLEY and GRELL (1968) and LEWIS (1978).

Culture media: (1) Gif standard medium ("GS"), which is, per liter of water, corn meal 100 g., agar 10 g, brewer's yeast 100 g and 50 ml of methyl-4-hydroxybenzoate in ethanol (1:9) and (2) Pasadena medium, *cf.*, LEWIS (1960).

RESULTS

Appearance of homeotic transformations following interactions between Df(1)sn *and* Df(3)red^{P93}

An important fraction of the progeny from *Df(1)sn/+* females display homeotic (metathorax → mesothorax) transformations when they also carry *Df(3) red^{P93}*. Missing metathoracic organs are infrequent (about 1%). The extent of the transformed territories is variable. Often, a part of a single metathoracic imaginal disc is transformed into the corresponding region of mesothorax. Both dorsal (haltere to wing) and ventral (third leg to second leg) discs are affected and both anterior and posterior compartments within them are affected; however, the anterior compartment is more frequently transformed. The presence of somewhat rarer transformations of prothorax to mesothorax was pointed out to us by P. INGHAM.

The detailed distribution of the various types of transformations was determined for only a small sample of *Df(3)red^{P93}/+* flies from *Df(1)sn/+* mothers (Table 1).

In the majority of the experiments to be described, only transformations readily observed under the dissecting microscope (16 ×) were taken into account, *e.g.*, ectopic thoracic structures in the metathorax, transformations of haltere to wing tissue and presence of sternopleural bristles (characteristic of the mesothorax) at the base of the third legs. In addition, the amplitude of the transformed tissue was not scored: a fly was classified simply as transformed or not transformed in the metathoracic regions just mentioned.

TABLE 1

Proportion of transformed structures in the progeny of Df(1)sn/M5 *females (a)* Df(1)sn/+; Df(3)red/+ *daughters, (b)* M5/+; Df(3)red/+ *daughters*

	PRO → MESO	META → MESO				Number of hemithoraces
	Leg 1	Nota	Halteres	Leg 3 (P)	Leg 3 (D)	
a	4%	11%	39%	27%	29%	220
b	4%	3%	21%	14%	12%	274

The deficient chromosome 3 was *Df(3)red^{P93}Sb*. Flies were grown at 23° and examined at 40× magnification. Transformed structures of the prothorax scored were the presence of apical or preapical bristles on the prothoracic leg. Presence of sternopleural bristles on the prothorax was not observed. Nota are ectopic dorsal structures in the metathorax. Transformations of the ventral parts of the metathorax scored were the presence of sternopleural bristles (P) and apical or preapical bristles (D) on the third leg. The transformations principally affected the anterior compartments.

Examination of Table 2 shows that among progeny of *Df(1)sn/+* females (crosses Ia and Ib), only those carrying *Df(3)red^{P93}* display transformations at a notable frequency. No transformed flies appear among progeny of *Df(1)sn/+* females, if the former carry two normal third chromosomes (cross Ib), and very few if they are heterozygous for *In(3L)P + In(3R)P18, Me Ubx e^{4}* (cross Ia). On the other hand, transformations are not confined to progeny bearing *Df(1)sn:* Flies not bearing *Df(1)sn* also show a high frequency of transformations, although somewhat lower than that of their deficiency-carrying sibs.

Crosses II and III yield progeny of genotypes analogous to those from cross Ia,

TABLE 2

Conditions under which the appearance of homeotic transformations occur as a result of interaction between Df(1)sn *and* (Df(3)red^{P93}

Cross	Maternal genotype	Genotype of zygotes					
		$\frac{X}{Df(1)sn};\frac{3}{3}$	$\frac{X}{X};\frac{3}{3}$	$\frac{X}{Y};\frac{3}{3}$	$\frac{X}{Df(1)sn};\frac{3}{Df(3)red}$	$\frac{X}{X};\frac{3}{Df(3)red}$	$\frac{X}{Y};\frac{3}{Df(3)red}$
Ia	$\frac{X}{Df(1)sn};\frac{3}{3}$	2/377	1/407	4/336	220/360	150/470	141/360
Ib	$\frac{X}{Df(1)sn};\frac{3}{3}$	0/368	0/549	0/544	——	——	——
II and III	$\frac{X}{X};\frac{3}{3}$	——	——	——	0/220	0/404	0/345

Cross Ia : *Df(1)sn/M5 × Df(3)red^{P93}, l(3)tr Sb/In(3L)P+In(3R)P18, Me Ubx e^{4}*
Cross Ib : *Df(1)sn/M5 × fs(1)h v^{24}*
Cross II: *M5/M5 × Df(3)red^{P93}, l(3)tr Sb/In(3L)P+In(3R)P18, Me Ubx e^{4}*
Cross III: *Df(3)red^{P93}, l(3)tr Sb/In(3L)P+In(3R)P18, Me Ubx e^{4} × Df(1)sn/Y; bw^{D}/ Dp(1; 2)FN107.*

X and *3* symbolize normal *X* and third chromosomes. In the case of the progeny of females not bearing *Df(1)sn* (crosses II and III), transformations were examined only in flies heterozygous for *Df(3)red.* In the bottom line, the zygotes *X/Df(1)sn; 3/Df(3)red* were derived from cross III, the two others from cross II. The number of transformed flies and the total number scored are recorded.

but from females not bearing *Df(1)sn*. Females and males from cross II are the same as *B Sb* females and *B* w^a *Sb* males from cross Ia; whereas, females phenotypically *bw Sb* from cross III have the same genotype as *Sb* females from cross Ia, except that one of the second chromosomes carried the marker bw^D. Other experiments have shown that the presence of this chromosome does not modify the frequency of transformations. It should be noted that no transformed flies appear among *Df(3)red*^{P93} progeny if their female parents did not carry *Df(1)sn*. This observation is contrary to that of CAPDEVILA and GARCIA-BELLIDO (1978).

The appearance of transformations seems therefore to rely simultaneously on the presence of *Df(3)red*^{P93} in the zygotes and the presence of *Df(1)sn* in the female parents.

Identification of the underlying genetic factors

The following experiments were designed to investigate whether the interactions previously described could be due, not to the deficiencies themselves, but rather to other genetic factors present on the deficient chromosomes.

Maternal effect of dosage of a gene situated in Df(1)sn: No other deficiencies of the *sn* region were available probably because of the presence of a nearby haplo-lethal zone (LEFEVRE and JOHNSON 1973). The duplication *Dp(1;2) FN107* (7A6 to 8A5), which covers *Df(1)sn*, was used to test the influence of dose of the region 7D1–7D5-6. *M5/Df(1)sn* females were crossed to *Df(1)sn/Y*; bw^D*/Dp(1;2)FN107* males. The resulting females, (*M5/Df(1)sn*; bw^D/+ and *M5/Df(1)sn*; *Dp(1;2)FN107*/+), were crossed to *Df(3)red*^{P93}, *l(3)trSb/ In(3L)P* + *In(3R)P18*, *Me Ubx* e^4 males. Phenotypically *Sb* flies from the two crosses were scored for the presence of transformations. The results are shown in Table 3.

No transformations were observed among the progeny of females carrying the duplication. It must be noted, however, that in this cross it is not possible to distinguish between descendants carrying the duplication and those not carrying it. It is possible that the presence, in the descendants, of the duplication could inhibit the transformations. The results from the two crosses are therefore not strictly comparable. Although other experiments have revealed that individuals

TABLE 3

The effect of Dp(1;2)FN107 *when present in* Df(1)sn/M5 *mothers*

Maternal genotype	Sex chromosomes of *Df(3)red*^{P93}/+ progeny		
	♀ *Df(1)sn*/+	♀ *M5*/+	♂ *M5/Y*
$\frac{Df(1)sn}{M5}$; $\frac{bw^D}{+}$	46* (202)	19* (234)	29* (209)
$\frac{Df(1)sn}{M5}$; $\frac{Dp(1;2)FN107}{+}$	0 (186)	0 (169)	0 (154)

Percentage of transformed flies among *Df(3)red*^{P93}/+ progeny from *M5/Df(1)sn* females with or without the duplication. The number of flies studied is indicated in brackets.

* The percentage of transformed flies was not statistically different among +/+ and +/bw^D progeny; the two groups have been pooled.

carrying *Dp(1;2)FN107* have a reduced viability, let us suppose that in the first cross there were as many *Dp(1;2)FN107*/+ as +/+ flies. The negative results obtained should therefore relate to half of the sample, *e.g.*, to 0/93 instead of 0/186 for the *Df(sn)*/+ females. Even with such a correction, where we surely overestimate the number of duplication-carrying flies, the experiment clearly indicates that the addition of the duplication into the maternal genome brings about a virtually complete suppression of the maternal effect of *Df(1)sn*. Thus, the effect can not be attributed to a genetic factor outside the duplication.

The preceding experiment is in agreement with dose effect in the female parent of the region 7D1–7D5-6, where two genes have been identified: *sn* and *fs(1)h*. A dosage effect of the sn^+ gene can be excluded; no transformed flies are found among *Df(3)red*^{P98}-bearing progeny of *sn* females. The $fs(1)h^+$ gene on the contrary, appears to be a good candidate since interactions have been observed between the temperature-sensitive allele *fs(1)h* and certain homeotic mutants (F. FORQUIGNON, in preparation). Indeed, *fs(1)h*/+ females crossed to *Df(3)red*^{P98}, *l(3)tr Sb/In(3L)P + In(3R)P18, Me Ubx* e^4 males give *Df(3)red*-carrying descendants, 10% to 20% of which are transformed when the cross is performed at 29°; no transformed flies are observed when the cross is performed at 23°.

Dose effect in the zygote of a gene situated in Df(3)red: Five deficiencies for *red* were used. They were of different origins and some were induced in rearranged third chromosomes. Males bearing these deficient chromosomes heterozygous for various balancer chromosomes were crossed to *M5/Df(1)sn* females. The results are shown in Table 4; only descendants carrying the deficient third chromosome are presented. Crosses in which the roman numerals are the same were performed simultaneously.

Examination of Table 4 shows that all five of these deficient third chromosomes have the same effect: flies carrying one of them and from *Df(1)sn*/+ female parents show a high frequency of transformations. It can also be seen that in each case the presence of *Df(1)sn* in the zygote is not required for the appearance of transformations, but nevertheless increases their frequency. The zygotic influence of dosage of the region 7D1–7D5-6 of the *X* chromosome will be presented later.

Closer examination of the different frequencies of transformation reveals some statistical differences between them. However, these differences are not linked to chromosome 3. When several third chromosomes are analyzed simultaneously, the frequencies are very similar (*e.g.*, Ib and Ic; IIa and IIb). At the moment, though, the distribution of the different types of transformations has not been analyzed.

The variations are mostly seen between crosses that were not performed simultaneously (*e.g.*, I and II). Mutant phenotypes of variable expressivity and penetrance are frequently seen to be highly sensitive to external factors and genetic background. We will try to analyze this subject later.

All of the *red* deficiencies used here include *Rg-bx*. This was seen by lethality of *Rg-bx/Df(3)red* flies. When mated to *Rg-bx/TM1* males, 14% of the

TABLE 4

Percentage of transformed flies heterozygous for various Df(3)red *chromosomes, in progeny of* Df(1)sn/M5 *mothers*

N° Exp.		Paternal genotype	Sex chromosomes of *Df(3)red/+* progeny ♀ *Df(1)sn/+*	♀ *M5/+*	♂ *M5/Y*
I	a	*Df(3)red^{1}/TM1*	68% ± 5	39% ± 5	47% ± 7
	b	*Df(3)red^{1}/DcxF*	68% ± 5	37% ± 5	48% ± 7
	c	*Df(3)red^{P93}, l(3)tr Sb/ In(3L)P+In(3R)P18, Me Ubx e^{4}*	61% ± 5	32% ± 4	39% ± 5
II	a	*Df(3)red^{1}/DcxF*	35% ± 5	16% ± 3	22% ± 5
	b	*Df(3)red^{P93}, l(3)tr Sb/ In(3L)P+In(3R)P18, Me Ubx e^{4}*	39% ± 4	15% ± 3	20% ± 4
	c	*Df(3)red^{91}/MRS*	42% ± 4	22% ± 3	20% ± 4
III	a	*Df(3)red^{1}/DcxF*	55% ± 8	29% ± 7	30% ± 7
	b	*Df(3)red^{P93}, l(3)tr Sb/ In(3L)P+In(3R)P18, Me Ubx e^{4}*	46% ± 7	19% ± 5	29% ± 6
IV	a	*Df(3)red^{P93}, l(3)tr Sb/ In(3L)P+In(3R)P18, Me Ubx e^{4}*	47% ± 7	20% ± 6	30% ± 6
	b	*Df(3)red^{31}/MRS*	52% ± 11	32% ± 8	25% ± 9
V	a	*T(2;3)ap^{Xa}Df(3)redP^{1}/TM1*	32% ± 11	13% ± 7	11% ± 8
	b	*T(2;3)ap^{Xa}Df(3)red^{P6}/TM1*	45% ± 12	23% ± 8	13% ± 7

The maternal genotype was *Df(1)sn/M5* in experiments I, II, III and V, and *Df(1)sn/M5; In(2)bw^{D}/+* in experiment IV. The percentages are accompanied by their standard errors.

Df(1)sn/+; Rg-bx/+ progeny were transformed. P. INGHAM (personal communication) also obtained a similar result, although the frequency of transformations that he observed was slightly higher. In addition, he also found a high frequency of transformed flies among descendants heterozygous for new lethal alleles of "*trx*," allelic to *Rg-bx* (INGHAM, personal communication). It is therefore most likely that the effect of *Df(3)red* is due to a dose effect of *Rg-bx^{+}* (or *trx^{+}*, according to which nomenclature is accepted), which has been supposed to intervene in segment determination (GARCIA-BELLIDO and CAPDEVILA 1978).

Influence of different factors on the frequency of transformed flies

Age of the female parent: Df(1)sn/M5 females were collected over a period of time from the same stock grown at 25°. They were aged for one day (sample A), nine to 10 days (sample B), 15 to 16 days (sample C) and 22 to 25 days (sample D), respectively. Females from samples B, C and D were kept at 25° on standard Gif medium ("SG"), periodically renewed to ensure proper nutrition. The five groups of females were all mated on the same day at 25° and cultured on identical media, to *Df(3)red^{P93}, l(3)tr Sb/In(3L)P + In(3R)P18, Me Ubx e^{4}* males. After a three-day egg-laying period, they were transfered without etherization to new bottles for another two days. The percentage of transformed heterozygous *Df(3)red* progeny was determined from this five-day egg laying period. The observed variation in frequency of transformed progeny does not

appear to be significant: A = 54%, B = 45%, C = 49% and D = 44%; $n > 250$ flies for each sample. The difference is of the same magnitude as that seen between flies of the same age.

Culture medium: The same cross was carried out at 25° on several different media: fresh GS medium, aged GS medium kept for eight days at room temperature, GS medium without moldex and the culture medium used at Pasadena. Bottles containing GS medium without Moldex developed a considerable amount of mold. On Pasadena food, flies developed more slowly than on GS food and viability of the different genotypes varies. Nevertheless, the different frequencies of transformed flies observed with the various media do not appear to be any greater than the differences seen between several repeats of the same test.

Temperature: Df(1)sn/M5 females grown at different temperatures were mated at hatching to *Df(3)red^{P93}, l(3)tr Sb/In(3L)P + In(3R)P18, Me Ubx e^4* males and allowed to lay eggs at the same temperatures, on identical media. The parental flies were periodically transferred to fresh media, and in spite of the fact that age of the females is not an important factor, the egg-laying periods were adjusted so that the progeny examined resulted from females of comparable physiological age.

The results, which are shown in Table 5, show that the frequency of transformed flies varies; it reaches a maximum at 23° and decreases at the extreme temperatures of 16° and 29°.

Genetic background: Examination of Table 4 reveals that the frequencies of transformed flies from crosses 1a and 1b, are very similar. The paternal genotypes, *Df(3)red^1/TM1* and *Df(3)red^1/DcxF*, had probably acquired quite different genetic backgrounds following breeding during the change of balancer chromosomes. In addition, crosses of various *Df(3)red/balancer* stocks of diverse origins, result in a similar frequency of transformations when they are performed simultaneously. This suggests a weak influence on the zygote of the genetic background.

In order to analyze the influence of both the genetic background and the structural rearrangement of the other homolog on the maternal effect of *Df(1)sn*, the following experiment was performed: *Df(1)sn/M5* females were mated to males of various genotypes. Daughters bearing *Df(1)sn*, associated with another

TABLE 5

Influence of temperature on the percentage of transformed flies

Temperature	Duration of egg laying	♀ *Df(1)sn/+*	♀ *M5/+*	♂ *M5/Y*
28.5° ± 0.5	5 days	24 (436)	9 (544)	9 (230)
25° ± 0.5	5 days	45 (399)	15 (461)	21 (360)
23° ± 0.5	7 days	56 (651)	31 (736)	35 (644)
20° ± 0.5	7 days	40 (551)	20 (542)	23 (432)
16° ± 0.5	13 days	30 (610)	14 (591)	16 (467)

The *Df(1)sn/M5* mothers and offspring were grown at the same temperature. Only *Df(3) red/+* progeny were examined. The table gives the percentage of flies transformed and the number examined (in parentheses).

X chromosome (whether or not this homolog was rearranged) and probably also bearing different autosomal backgrounds, were crossed to sn^3; $Df(3)red^{p93}$, *l(3)tr Sb*/+ males. The presence of sn^3 permits identification of *Df(1)sn*/+ female progeny in all of the crosses. The results are presented in Table 6.

Apparently, the maternal effect of *Df(1)sn* does not depend upon the presence or absence of structural rearrangements in the *X* chromosome partner, nor is it very sensitive to the autosomal genetic background. Apart from the last maternal genotype studied [*Df(1)sn/cm*] the observed variations are no greater than those observed between repeats of the same cross. In the case of *Df(1)sn/cm* female parents, the percentage of transformed progeny is much higher; these high values have also been observed in other experiments. At present, we have not analyzed the genetic factor responsible.

We conclude that among the external factors studied, only temperature appears to have a distinct effect. The temperature dependence, however, cannot be responsible for the variations sometimes observed when the same cross is repeated at 25 ± 0.5° over intervals of several months. Therefore, as long as the causal factor has not been identified, it is useful to systematically introduce a reference cross with each experiment.

Zygotic effect of dosage of the region 7D1–7D5-6

In all the preceding tables, it will be noticed that the percentage of transformed flies is twice as high in *Df(1)sn*/+; *Df(3)red*/+ females as in their +/+; *Df(3)red*/+ female sibs. Presumably because of the phenomenon of dosage compensation of *X*-linked genes in the male, the frequency of transformations in the latter is of the same order of magnitude as in the females of the second type, although it is systematically slightly higher. The effect of introducing a third dose of the region 7D1–7D5-6 has been studied in the following experiment: *Df(1)sn/M5* females were crossed to *Df(1)RA2*; *Dp(1;2)FN107/bw*^{D}; $Df(3)red^{p93}$, *l(3) tr Sb*/+ males. *Df(1)RA2* does not include the 7D1–7D5-6 region. The presence of *Df(1)RA2* was found to be necessary, due to the almost total sterility of males carrying a normal *X* chromosome and duplication *FN107*. The percentage of transformations in $Df(3)red^{p93}$, *l(3)tr Sb*/+ flies carrying

TABLE 6

Influence of different X *chromosomes homologous of* Df(1)sn, *on the frequency of transformed* Df(3)red/+ *progeny*

Maternal genotype	Sex chromosomes of *Df(3)red*/+ progeny		
	♀ *Df(1)sn*/+	♀ *Bal**/+	♂ *Bal**/*Y*
Df(1)sn/M5	57 (295)	18 (356)	22 (298)
Df1()sn/asc	62 (185)	26 (242)	26 (207)
Df(1)sn/FM6	47 (329)	24 (360)	25 (301)
Df(1)sn/FM4	48 (351)	21 (414)	22 (284)
$Df(1)sn/v^{24}$	44 (272)	28 (442)	27 (367)
Df(1)sn/cm	79 (315)	56 (533)	50 (525)

* *Bal*: Balancer, or other maternal *X* chromosome.

one, two or three doses of the region 7D1–7D5-6 is indicated in Table 7. The presence of three doses of the wild-type allele of the *fs(1)h* gene diminishes, but does not completely abolish the number of transformed flies. The effect of four doses of the gene would be interesting to study since it would enable us to resolve the function linking the frequency of transformations with gene dosage. Such an experiment is difficult to perform since a necessary condition for the appearance of transformations is the presence of only one dose of $fs(1)h^+$ in the female parent, which means that three doses would have to be supplied by the male parent.

One sees that the frequency of transformations is high among *Df(1)sn; Dp(1;2)FN107/+* males. If this result is confirmed, then it suggests that dosage compensation is not applied to $fs(1)h^+$ when this gene is translocated to an autosome.

Interaction in the female parent between Df(1)sn *and* Df(3)red

A maternal effect of *Df(3)red* has been reported by CAPDEVILA and GARCIA-BELLIDO (1978) and also for *trx* (INGHAM 1980). It therefore seemed worthwhile to investigate the association of these two deficiencies in the female parent to see whether this would increase the frequency of transformations among *Df(3)red/+* progeny.

Df(1)sn/M5 females were crossed to *M5; Df(3)red^1/DcxF* males. The resulting *Df(1)sn/M5; Df(3)red^1/+* females and *Df(1)sn/M5; DcxF/+* females were crossed to *Df(1)red^{p93}, l(3)tr Sb/In(3L)P+In(3R)P18, Me Ubx e^4* males. A cross between *Df(1)sn/M5* females and the same males was performed simultaneously. The percentage of transformed flies among the *Df(3)red^{p93}, l(3)tr Sb/+* progeny from all three crosses was determined (Table 8).

The presence of *Df(3)red^1* in the female parents does not appear to modify the frequency of transformation. On the contrary, the presence of the *DcxF* chromosome 3 seems to increase considerably the maternal effect of *Df(1)sn*. This chromosome, however, has no breakpoints near either *Rg-bx* or the bithorax

TABLE 7

Zygotic effect of dosage of the region 7D1–7D5-6

Genotype of progeny for X and 2 chromosomes	Number of 7D1–7D5-6 regions	% flies transformed
Df(1)sn/Df(1)RA2; bw^D/+	1	50 (105)
Df(1)sn/Y; Dp(1; 2)FN107/+	1	45 (60)
Df(1)RA2/M5; bw^D/+	2	29 (134)
Df(1)sn/Df(1)RA2; Dp(1; 2)FN107/+	2	32 (155)
Df(1)sn/M5; Dp(1; 2)FN107/+	3	15 (170)

M5/Df(1)sn females were crossed to *Df(1)RA2; bw^D/Dp(1; 2)FN107; Df(3)red^{p93}, l(3)tr Sb/+* males at 25°. Only progeny bearing *Df(3)red* were scored. *M5; bw^D/+* males (one dose) and *M5; Dp(1; 2)FN107/+* males (two doses) are not shown because it is not easy to distinguish a *bw* phenotype in *B w^a* flies; the percentage of transformed *M5* males bearing *bw^D* or *Dp(1; 2)FN107* was 26% (226).

TABLE 8

Interaction between Df(1)sn *and* Df(3)red *at the level of the maternal genotype*

Maternal genotype	Sex chromosomes of *Df(3)red^{P93}Sb*/+ progeny		
	♀ *Df(1)sn*/+	♀ *M5*/+	♂ *M5*/*Y*
$\frac{Df(1sn)}{M5}; \frac{+}{+}$	43 (396)	14 (411)	26 (408)
$\frac{Df(1)sn}{M5}; \frac{Df(3)red^{1}}{+}$	48 (163)	15 (329)	24 (152)
$\frac{Df(1)sn}{M5}; \frac{DcxF}{+}$	79 (42)	66 (59)	65 (34)

The percentages of transformed flies are accompanied by the number of flies (in brackets).

region. This effect, however, needs to be further verified since the sample was small.

Interaction between Df(1)sn *and genes, other than* Rg-bx, *which may be implicated in the process of segmental determination*

The results obtained to date are only preliminary; nevertheless, they show that *Df(1)sn* does not interact solely with *Df(3)red*.

Table 9 shows the percentages of transformed individuals among flies from *Df(1)sn* females that have acquired various third chromosomes from the father. A high frequency of transformations is seen among flies carrying *ru Rg-pbx e^{s}ca*; whereas no transformation was observed in 450 descendants of *M5/M5* females. *Rg-pbx* (LEWIS 1968) is a homozygous lethal and dominant mutation causing

TABLE 9

Percentage of transformed flies from Df(1)sn/M5 *mothers and bearing different paternally derived third chromosomes*

Nature of the paternal third chromosome ("*3p*")	Genotype of progeny		
	$\frac{Df(1)sn}{X}; \frac{3}{3p}$	$\frac{X}{X}; \frac{3}{3p}$	$\frac{X}{Y}; \frac{3}{3p}$
+	0 (368)	0 (549)	0 (544)
Sb	0,5 (605)	0,2 (586)	0,2 (534)
su-Hw^{2} bx bxd	0 (276)	NE	NE
bx pbx	0 (200)	NE	NE
ru Rg-pbx e^{s} ca	35 (577)	9 (497)	14 (429)
In(3L)P+In(3R)P18, Me Ubx e^{4}	0,5 (377)	0,2 (407)	1,2 (336)
T(2; 3)ap^{Xa}	0 (126)	0 (131)	0 (113)
TM1	2 (302)	NE	NE
MRS	0,6 (174)	0 (178)	0 (137)
DcxF	2,7 (973)	0,9 (1526)	2,2 (974)
TM 2(In(3LR)Ubx^{130})	13,6 (800)	3,5 (824)	7,9 (763)
In(3R)Ubx^{80}	10,8 (332)	3,7 (381)	7 (393)
Df(3)P9	13,9 (324)	3,3 (421)	4,2 (283)

NE: Not examined; *3p*: paternal third chromosome.

postbithorax-like transformations. The penetrance varies in different stocks and was very weak in the *ru Rg-pbx e^{s}ca/TM1* stock used here, which probably explains the absence of transformations in the control cross of *M5/M5* ♀ × *ru Rg-pbx e^{s}ca/TM1* ♂. The observed transformations carried by *ru Rg-pbx e^{s}ca* flies can affect both the anterior and posterior compartments of the metathorax. However a comparative study of the extent of the transformed areas in *Rg-pbx/+* and of *Df(3)red/+* or *Rg-bx/+* has not been performed.

Transformations, albeit less frequent, are also found among descendants heterozygous for *Ubx^{130}*, *Ubx^{80}* and *Df(3)P9*, a deficiency including the whole of the bithorax region. In contrast, they appear only exceptionally among descendants heterozygous for *Ubx^1* or for the recessive mutations of the bithorax region that have been tested. Lastly, it should be pointed out that one of the rearranged third chromosomes, *DcxF*, appears to interact weakly in the zygote with *Df(1)sn* in spite of the fact that it bears no breakpoint near bithorax or *red*.

DISCUSSION

The experiments just described show the existence in region 7D1–7D5-6 of the *X* chromosome of a gene implicated in segment determination. Most probably the gene concerned is *fs(1)h*, a heat-sensitive allele of which has been isolated in our laboratory (FORQUIGNON, in preparation). It is possible that we have rediscovered a gene described by WADDINGTON (1956). This author, following a continuous selection of flies showing bithorax phenocopies after ether treatment at blastoderm, obtained a stock in which the homeotic transformations appeared *in the absence of any treatment*. Studies of this stock revealed that "the bithorax phenotype is dependent on two components: a collection of genes, located on all the chromosomes, which act directly on the individual containing them, and a genetic condition of the first chromosome which causes a maternal effect" (WADDINGTON 1956). The second component was localized close to vermilion, which, given the degree of precision of the experiments, is not incompatible with the position of *fs(1)h* 10 crossover units to the left of vermilion.

Current models concerning the determination process postulate the existence of positional signals that would be under the control of genes that have yet to be uncovered. Some of the properties of *fs(1)h* suggest that this gene may well play a role at this level and represent one of the missing elements of the system. However, its role is far from clear.

Two arguments favor the participation of *fs(1)h* in the establishment of positional information. The first is the important maternal effect of hypoploidy of region 7D1–7D5-6 in which the gene is located. The second is the analogy seen between the effect of maternal hypoploidy of *fs(1)h* and that of ether treatment of blastoderm stage embryos (GLOOR 1947; CAPDEVILA 1977; BOWNES and SEILER 1977; CAPDEVILA and GARCIA-BELLIDO 1978). The mutations (or deletions) causing the appearance of homeotic transformations among the descendants of *Df(1)sn/+* females are the same as those causing an increase in the frequency of ether-induced phenocopies: *Rg-bx* [*or Df(3)red*], *Rg-pbx*, *Ubx^{130}*, *Ubx^{80}*,

Df(3)P9, but not *Ubx*^{1} or any of the recessive mutations of the bithorax system that have been studied. Furthermore, the relative efficiencies of the mutations are similar in the two systems: *Rg-bx* and *Rg-pbx* are more effective than *Ubx*^{1so}, *Ubx*^{so} or *Df(3)P9*. Also, the types of transformations are very similar: partial transformations are more frequent in the anterior than in the posterior compartment. Moreover, the frequency of ether-induced phenocopies among the progeny of *Df(1)sn*/+ females is higher than that seen in the progeny of wild-type flies (SANTAMARIA, personal communication). If, as has been suggested (CAPDEVILA and GARCIA-BELLIDO 1978), ether treatment perturbs the positional information present in the cortex of the egg, the analogy between hypoploidy of *fs(1)h* in the female parents and ether-induced phenocopies is an argument in favor of a role of *fs(1)h* in the establishment of such positional signals. However, even though ether vapors are maximally effective at precisely the time when segment determination is believed to occur (SANTAMARIA 1979), its mode of action is still too poorly understood for the argument to be a strong one.

Furthermore, the dosage of *fs(1)h* also has a zygotic effect: among *Df(3) red*/+ progeny of *Df(1)sn*/+ females, the frequency of transformed flies is inversely proportional to the doses of the wild-type allele of *fs(1)h*. This relationship brings to mind the dose effect of the bithorax region on the frequency of ether-induced phenocopies (CAPDEVILA and GARCIA-BELLIDO 1978). The zygotic effect of *fs(1)h* is of less importance than the maternal effect in the sense that homeotic transformations appear even in the presence of three doses of *fs(1)h*$^{+}$; whereas, in the female parent, two doses of *fs(1)h*$^{+}$ are sufficient to eliminate them. Nevertheless, the zygotic effect is difficult to explain in the framework of the hypothesis evoked earlier, unless one supposes that the positioning of the informational molecules is not completed at the time of egg laying (SANDER 1975; SCHUBIGER 1976). Another, very hypothetical, postulate would be to attribute a different role to *fs(1)h* in the female parent from that in the zygote: participation in the establishment of a positional information at the maternal level and competition, at the zygotic level, between *fs(1)h* and the bithorax region for a regulatory substance/repressor in the models presented by LEWIS (1978) and GARCIA-BELLIDO and CAPDEVILA (1978).

A two-fold action, zygotic and maternal, is a property also ascribed to *Df(3)red* (CAPDEVILA 1977; CAPDEVILA and GARCIA-BELLIDO 1978), as well as for new recently isolated alleles of *Rg-bx* (INGHAM 1980; INGHAM and WHITTLE 1980). Furthermore, the most important synergistic action observed is that between *Df (1)sn* and *Df(3)red*. This suggests that *fs(1)h* and *Rg-bx* may act in two steps of the same process or in two parallel redundant processes, the existence of which would be justified by the importance of segment determination. In the experiment described, the role of dosage of the two genes appears quite dissimilar: that of *fs(1)h* acts principally at the level of the maternal genotype; whereas, that of *Df(3)red*, under our experimental conditions and with the stocks used, appears to act only at the zygotic level. Even when associated with *Df(1)sn*, no maternal effect of *Df(3)red* was detected. If one postulates that *fs(1)h* and *Rg-bx* are redundant genes, both participating, as has been suggested for *Rg-bx* (GARCIA-

BELLIDO and CAPDEVILA 1978), in the establishment of positional information, then one would have to suppose that they have diverged, as far as their time of action is concerned, *fs(1)h* acting mainly during ovogenesis, *Rg-bx* mainly at the beginning of embryonic development.

It is obvious that much information is lacking concerning the role of *fs(1)h* in segment determination. Particular care has been taken to define the influence of culture conditions and genetic background in the expression of the interactions between *Df(1)sn* and *Df(3)red* because of the incomplete penetrance of the phenotype. The frequency of transformed flies is quite stable, a condition favorable for further analysis of the more complex interactions involving, in addition to the dosage of *fs(1)h* and *Rg-bx*, mutations or dosage effects of other genes implicated in the activation of selector genes, *Rg-pbx*, *Pc* and the regulatory zones of the bithorax region. It was also of importance to define the interactions between null alleles (or deficiencies) of *fs(1)h* and *Rg-bx* in order to be able later to interpret interactions between hypomorphic and heat-sensitive alleles of the two genes. Apart from the further studies just described, the synergism between the effect of hypoploidy of *fs(1)h* and *Df(3)red* open other experimental possibilities, namely, the *red* deficiencies could be used to uncover other unidentified genes that may have an effect analogous to that of *fs(1)h*. This would enable us to define the particularly important point of the degree of specificity of *fs(1)h*.

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