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Mobilization of the *gypsy* and *copia* retrotransposons in *Drosophila melanogaster* induces reversion of the *ovo*^{D} dominant female-sterile mutations: molecular analysis of revertant alleles

Maryvonne Mével-Ninio[1], Marie-Christine Mariol and Madeleine Gans

Centre de Génétique Moléculaire du CNRS, 91190 Gif-sur-Yvette, France

[1]Temporary address: Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, MA 02138, USA

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The *ovo* locus is required for the maintenance of the female germ line in *Drosophila melanogaster*. In the absence of an *ovo*$^+$ gene, males are completely normal but females have no germ-line stem cells. Three dominant mutations at the *ovo* locus, called *ovo*^{D}, were observed to revert towards recessive alleles at high frequency when *ovo*^{D} males were crossed to females of the strain *y v f mal*. We have found that this strain contains an inordinately high number of *gypsy* transposable elements, and crossing it with the *ovo*^{D} strains results in the mobilization of both *gypsy* and *copia*, with high-frequency insertions into the *ovo* locus: of 16 revertants examined 12 have *gypsy* and four have *copia* inserted at 4E, the *ovo* cytological site. Using *gypsy* DNA as a tag we have cloned 32 kb of wild-type DNA sequences surrounding a *gypsy* insertion and characterized molecular rearrangements in several independent revertants: in 10 of them *gypsy* appears to be inserted into the same site. The orientation of *gypsy* is strictly correlated with whether the neighbouring *lozenge-like* mutation appears in the revertants. A distal limit of the *ovo* locus was molecularly determined from the breakpoint of a deletion affecting closely flanking regions.

Key words: Drosophila/ovo^D reversion/*gypsy* mobilization/*shavenbaby*

Introduction

In *Drosophila melanogaster* a large number of mutations have been described that result in female sterility. Most of these mutations are pleiotropic. Among the mutants specifically affected in oogenesis, many different developmental processes may be altered. As somatic cells and germ cells are both necessary for the production of eggs, female-sterile mutations could *a priori* affect either one, or both of these cell lineages. However, few mutations have their effects restricted to the early female germ-line development. Among the latter are the female-sterile mutations identified at the X-linked *ovo* locus (Busson *et al.*, 1983). Female embryos homozygous for a 'loss of function' allele of *ovo* exhibit early degeneration of pole cells (Oliver *et al.*, 1987). Males carrying *ovo* loss of function alleles are indistinguishable from wild-type males in their viability and fertility, indicating that the *ovo*$^+$ function is specific to the female germ-line. While most known female-sterile mutations are recessive, the first three mutations identified at the *ovo* locus, called *ovo*^{D}, were dominant. Females heterozygous for the strongest allele, *ovo*^{D1} (hereafter called *D1*), fail to lay eggs and have markedly atrophied ovaries. The *D1* allele is dominant to such an extent that females remain sterile even when they carry three doses of the wild-type gene.

While characterizing the *ovo*^{D} mutations, Busson *et al.* (1983) observed that crossing females of the strain *y v f mal* to *ovo*^{D} males resulted in a high frequency of reversions which led to loss of function (*ovo*^{r}) alleles. This phenomenon was not observed when females of other strains were crossed to *ovo*^{D} males. In some cases, the reversions were associated with the appearance of mutations at other genetic loci: both lethal mutations and mutations with a visible phenotype, in particular *cut* (*ct*), were observed. These characteristics suggested that crossing *ovo*^{D} males with the *y v f mal* strain results in the mobilization of one or several transposable elements, with the *ovo* locus constituting a hot spot for insertions. Neither the *P-M* nor *I-R* dysgenic systems appeared to be implicated, since the strains used in the crosses presented an *M-I* cytotype and did not hybridize with *P* (D.Busson and M.Gans, unpublished data).

We have been interested in determining which transposable element is responsible for the observed reversions for several reasons. First, we hoped that identifying that element would help us to clone the *ovo* locus. Second, except for the *P*, *I* and *hobo* elements, whose mobilization has been thoroughly studied (Bregliano and Kidwell, 1983; Rubin, 1983; Bucheton *et al.*, 1984; Blackman *et al.*, 1987; Yannopoulos *et al.*, 1987) little is known of the conditions under which the *Drosophila* transposable elements become active: by taking advantage of the *y v f mal* strain it might be possible to study the regulation of the element(s) involved in the reversion phenomenon.

We now show that crosses with the *y v f mal* strain result in the mobilization of the *copia* and *gypsy* retrovirus-like elements. All the examined *ovo*^{D} revertants issued from a cross with the *y v f mal* strain have acquired either a *gypsy* or a *copia* element in 4E, the cytological site of the *ovo* locus. We have cloned wild-type DNA sequences surrounding one of these *gypsy* inserts, and molecularly characterized the rearrangements undergone by the *ovo* locus in a number of *ovo*^{D} revertants.

Results

Cytogenetic mapping of ovo^D

The *ovo* locus was originally mapped to cytological position 4D-E on the X chromosome (Busson *et al.*, 1983). Its location was narrowed down to position 4E1-2 by Oliver *et al.* (1987). Complementation analysis of *ovo* and of the closely flanking mutation *shavenbaby* (*svb*; Wieschaus *et al.*,

1984) with a set of pre-existing deficiencies (Table I) shows that *ovo* is located proximally to *svb*. Of immediate interest is the deletion $Df(1)bi^{D2}$ which retains the wild-type function of *ovo* but does not complement *svb*; the proximal end of $Df(1)bi^{D2}$ provides us with a distal boundary of the *ovo* locus. *Ovo* and the closely flanking mutation *rugose* (*rg*) are removed by the same deficiencies. We were able to show that *rg* is located proximally to *ovo* by taking advantage of strain *Q259* in which a *rg* mutation has been induced by the insertion of a *white* transposon (Hazelrigg *et al.*, 1984); hybridization of a biotinylated *white* probe on the salivary gland chromosomes showed that the insertion of the transposon has taken place at the 4F1,2 cytological site.

Spontaneous reversions of ovo^D

The three dominant ovo^D alleles *D1*, *D2* and *D3* revert spontaneously towards recessive female sterility at high frequencies when ovo^D males are mated to females of the strain *y v f mal*. The recessive alleles, ovo^r, behave as deficiencies of the wild-type allele; ovo^r/ovo^r females show the same extreme atrophy of their ovaries as the ovo^r/Df ones (Busson *et al.*, 1983). We have made a series of crosses in order to define the genetic characteristics of the reversion phenomenon. When homozygous *y v f mal* females are crossed to *D1* males, the fraction of *y v f mal/D1* daughters with a reversion event in the germ-line is $\sim 2 \times 10^{-2}$ (Figure 1; this value is somewhat less than the one reported by Busson *et al.* in 1983 which was 6×10^{-2}). The frequency of reversion drops to 10^{-4} when *D1* males are crossed to females homozygous for the X-chromosome balancer *M5*. This last value is in agreement with those reported by Oliver *et al.* (1987): 3×10^{-5} in a *FM3* genetic background and 6×10^{-4} after gamma-ray irradiation. These observations suggest that the *y v f mal* strain is responsible for the high frequency of reversion, which is not a property of the *D1* strain. When heterozygous *M5/y v f mal* females are crossed to *D1* males (crossing schemes III and IV in Figure 1), the frequency of reversion is the same in the *M5/D1* and *y v f mal/D1* daughters, suggesting that the X chromosome carrying the *y v f mal* markers is not essential in the reversion process. Reciprocal crosses generating *M5/y v f mal* females have been performed, in order to determine whether maternal inheritance from the *y v f mal* strain is important. From the results reported in Figure 1 it can be seen that the frequency of reversion is approximately the same in the *M5/D1* and *y v f mal/D1* females issued from one or the other reciprocal cross. Based on these results it is reasonable to assume that the factors responsible for the reversion phenomenon are not cytoplasmically inherited. They are more likely located on the chromosomes of the *y v f mal* strain, and not exclusively on the X chromosome.

Table I. Cytogenetic localization of *ovo*

Nomenclature	Extent	Complementation with	
		ovo^r	*svb*
$Df(1)bi^{D1}/FM7c^a$	4B3,4-4D1,2	+	+
$Df(1)bi^{D2}/FM7c^a$	4B6,C1-4D7,E1	+	−
$Df(1)rb^{32}/FM6^a$	4A6,B2-4E2,F1	−	−
$Df(1)RC40/FM7a^b$	4B1-4F1	−	−

[a]Banga *et al.* (1986); [b]Pasadena stock center.

Effect of P-M hybrid dysgenesis on reversion of D1

We attempted to obtain reversion of *D1* using *P-M* hybrid dysgenesis (Table II). The frequency of reversion in *M5/D1* females issued from dysgenic crosses remained very low (3×10^{-4} at most) and was not significantly above that observed in control non-dysgenic crosses. In the six dysgenically induced revertants examined (lines 72, 81, 90, 91, 93 and 101), no *P* element was visible in the 4E region of salivary gland chromosomes, where the *ovo* mutation has been mapped. Dissection revealed that, with the exception of line 72, the $ovo^D/+$ females that had given rise to revertant lines bore only one functioning ovary, indicating that reversion took place in the germ-line of these $ovo^D/+$ females. In the case of line 72 two functional ovaries were seen, suggesting that reversion may have taken place in a male germ-line cell of the *D1/Y* father. In one set of experiments, dysgenic crosses were performed using *y v f mal* females. They resulted in no observable change in the frequency of reversion.

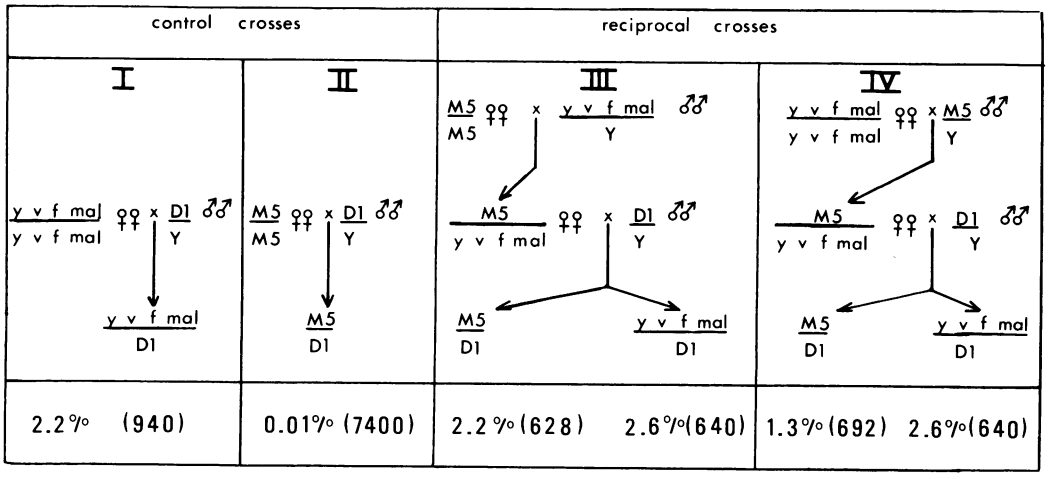

Fig. 1. Sequences of crosses used to compare the frequency of reversion of *D1* in various genetic contexts. Numbers in the last line represent the percentage of heterozygous *D1* females laying fertile eggs. In brackets is the number of screened females.

Mutations associated with reversions of ovo^D

Two mutations closely linked to the *ovo* locus have been frequently observed in revertant lines. One of these is a lethal (*le*) mutation with partial penetrance. The few *le ovo^r* males that survive show a phenotype characterized by lightly curved bristles and unspread wings. We have shown that this lethal mutation is an allele of *svb*: (i) the *svb* phenotype is visible in *le ovo^r* male larvae, which have a reduced number of denticle belt setae; (ii) when heterozygous *svb^{YD39}/FM7* females are mated to *le ovo^r* males, the hatching *svb^{YD39}/le ovo^r* daughters exhibit the typical bristle and wing defects shown by the *le ovo^R* males. It may be noted that the *svb* mutation is present in almost all the revertants obtained in a genetic background different from the *y v f mal* one (see Table II; six out of seven revertants obtained in the *M5* context are *svb*). A similar observation was reported by Oliver *et al.* (1987) who, out of 24 revertants, obtained 22 *svb* mutants, following gamma-ray or EMS mutagenesis in a *FM3* genetic background. In the *y v f mal* context only a small fraction (~3%) of the revertants are *svb*, except in the case of hybrid dysgenic crosses (see Table II; the three revertants issued from dysgenic crosses performed with *y v f mal* females are *svb*).

The other mutation that often accompanies reversion of the *D1* mutation and is closely linked to it typically has a *lozenge-like* eye phenotype (*lzl*). Flies with an extreme *lzl* phenotype exhibit eyes whose surface is completely smooth. Up to now the *lzl* mutation has been observed only in crosses involving the *y v f mal* strain, but is present in 5–25% of the revertants. It is associated with reversion of the strongest *D1* allele, but not with that of the *D3* allele. Expression of the *lzl* phenotype is cold sensitive, being stronger at 18°C than at 25°C, and it is semi-dominant. Using meiotic recombination experiments we have mapped *lzl* between the visible markers *ec* and *cv* at <0.5 crossing-over units from the *ovo* locus.

Table II. Effect of *P-M* hybrid dysgenesis on reversion of *D1*

Homozygous mothers	Fathers	Number of daughters screened	Number of revertants and characteristics	Allele
M5 (M)	*D1* (P)	6200	2 *svb*	81,82
M5 (M)	*D1* (M)	6800	1 *svb*	80
M5 (P)	*D1* (HD)	3000	1	72
M5 (M)	*D1* (HD)	3500	1 *svb*	71
M5 (P)	*D1* (HD)	10 000	2 *svb*	101,104
M5 (P)	*D1* (M)	11 000	0	
y v f mal (M)	*D1* (P)	1000	3 *svb*	90,91,93
y v f mal (M)	*D1* (M)	1000	4	85,86,88,89

The letters in brackets, M and P, indicate the cytotype of the strain used in the cross. HD indicates that the individuals were issued from a stock in which *P-M* hybrid dysgenesis had been induced (see Materials and methods).

Table III. Revertants of ovo^D

Allele[a]	Reversion context	Associated mutations	Presence at 4E of	
			gypsy	*copia*
ovo^{D1rS14}	*y v f mal*	*lzl*	+	–
ovo^{D1rS22}	*y v f mal*	*lzl*	+	–
ovo^{D1rS32}	*y v f mal*	–	+	–
ovo^{D1rS35}	*y v f mal*	*lzl*	+	–
ovo^{D1rS36}	*y v f mal*	–	+	–
ovo^{D1rS37}	*y v f mal*	–	+	–
ovo^{D1rS38}	*y v f mal*	*lzl*	+	–
ovo^{D1rS78}	*y v f mal*	*lzl*	+	–
$ovo^{D1rS124}$	*y v f mal*	*lzl*	+	ND
ovo^{D3rS54}	*y v f mal*	–	+	–
ovo^{D3rS57}	*y v f mal*	–	+	–
ovo^{D3rS58}	*y v f mal*	*cut*	+	–
ovo^{D3rS59}	*y v f mal*	–	–	+
$ovo^{D1rHD90}$	*y v f mal* + HD[b]	*svb*	–	+
$ovo^{D1rHD91}$	*y v f mal* + HD	*svb*	–	+
$ovo^{D1rHD93}$	*y v f mal* + HD	*svb*	–	+
$ovo^{D1rHD72}$	HD	–	ND	–
$ovo^{D1rHD81}$	HD	*svb*	–	–
$ovo^{D1rHD101}$	HD	*svb*	–	–

[a]In the text, for convenience, revertants are referred to as numbers: ovo^{D1rS14} = 14.
[b]HD, Hybrid dysgenic cross.
In addition, the five revertants 31, 51, 118, 119 and 121, which we have molecularly characterized and which have been obtained in diverse genetic contexts, were associated with *svb*. They were maintained in *FM7c* balanced stocks, and therefore have not been cytologically scored for *gypsy* or *copia* insertions.

Presence of a transposable element in revertants of ovo^D

We looked for presence of a transposable element in the three dominant alleles, *D1*, *D2* and *D3*, and in 19 independent revertant strains issued from *D1* and *D3* (Table III). For each strain and transposable element we performed *in situ* hybridization on salivary gland chromosomes of 3–5 male larvae. None of the tested elements (*P, I, 297, copia* and *gypsy*) was found at the *ovo* cytological site in the three dominant alleles. By contrast, out of 16 revertants obtained in the *y v f mal* context, 12 possess a *gypsy* hybridization site at 4E, the cytological site of *ovo*, and the four remaining ones have *copia* sequences at the same site. Of the latter, three are issued from *P-M* dysgenic crosses performed with *y v f mal* females and are associated with *svb*. On the other hand, neither *gypsy* nor *copia* were found at 4E in the three lines, 72, 81 and 101, issued from *P-M* dysgenic crosses performed with *M5* females.

In order to make sure that the presence of the *gypsy* or *copia* element at 4E in the revertants did not result from crossing over involving the X chromosome of the *y v f mal* strain, it was necessary to investigate the distribution of *gypsy* and *copia* in the latter strain. Concerning *copia*, in the 10 *y v f mal* larvae studied, 26–28 euchromatic sites were observed, with four sites on the X chromosome, at 1A, 1B, 5A and 5C; none of these sites exists in the *D1*, *D3* and revertant strains. Examination of the *y v f mal* strain for its content of *gypsy* elements (12 larvae examined) revealed no *gypsy* inserts at 4E, in spite of 45–65 euchromatic hybridization sites (Figure 2C), including ~20 sites on the X chromosome. Such a high number of *gypsy* elements is very unusual in *D.melanogaster*, which has been reported to contain an average number of five *gypsy* insertions on the chromosome arms (Modolell *et al.*, 1983). The *D1* and *D3* strains contain only two and three *gypsy* respectively, but none on the X chromosome (Figure 2A). On the X chromosome of revertant strains a variable number of *gypsy* elements – from one to seven – was observed (Figure 2B; 15

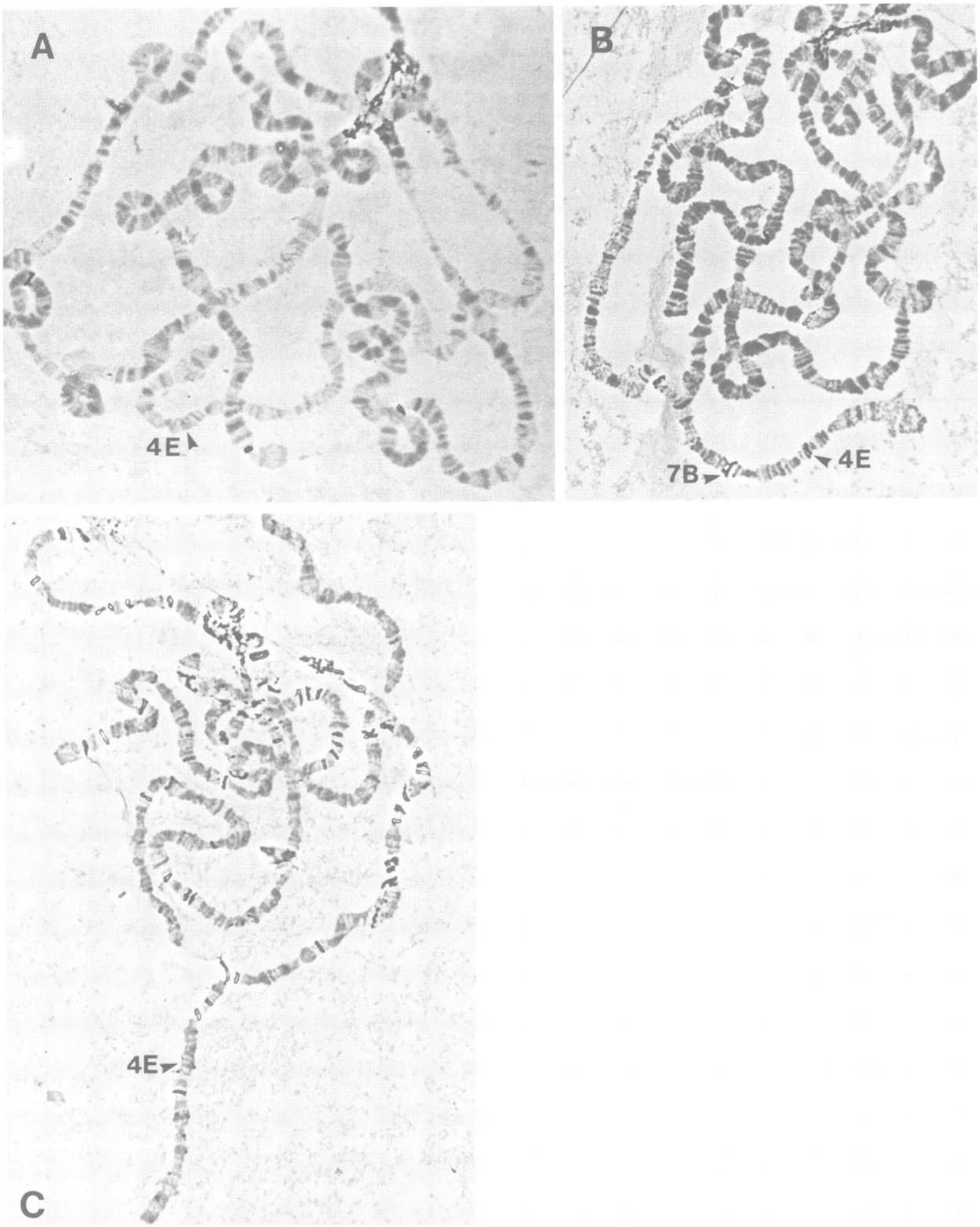

Fig. 2. (**A**) Chromosomes of *D3*; (**B**) chromosomes of the revertant line 58 issued from *D3*; (**C**) chromosomes of the *y v f mal* strain. Hybridization was performed with the biotinylated clone cDm111 (Bayev *et al.*, 1984) containing the full-length *gypsy* element. Arrowheads mark the location of the 4E and 7B bands. Hybridization at 4E and 7B is seen in (B) on the X chromosome of line 58 in which both ovo^r and *ct* mutations appeared.

lines studied and 11 diagrammed in Figure 3A). In revertant 58, in which the additional mutation *ct* appeared, a hybridization site was visible at 7B, the cytological site of *ct*.

The distribution of the *copia* element on the X chromosome of 18 revertants (17 diagrammed in Figure 3B) was compared to that observed in the *D1* and *D3* strains from which these revertants originated. *D1* and *D3* were obtained in two different lines of the v^{24} strain that had each been isogenized for the X chromosome. Within *D1* or *D3* the localization of *copia* was found to be invariant,

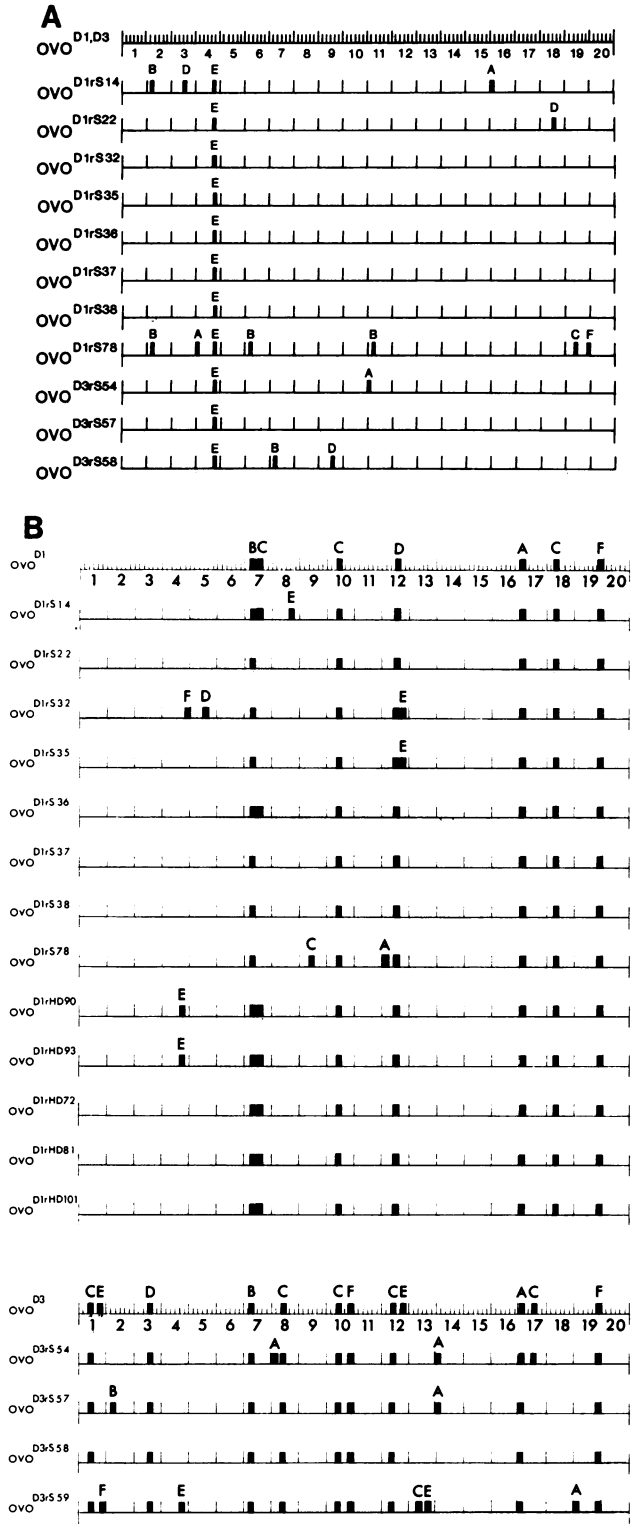

Fig. 3. (**A**) Location of the *gypsy* element insertion sites on the X chromosome of eight revertants issued from *D1* and of three revertants issued from *D3*. Revertant lines 90, 91, 93, 59, 81 and 101 have been cytologically scored but are not illustrated since they do not contain *gypsy* insertions on their X chromosome. Revertant line 72 has not been cytologically scored for *gypsy* insertions. (**B**) Location of the *copia* element insertion sites on the X chromosome of 13 revertants issued from *D1* and in four revertants issued from *D3*. The pattern of *copia* insertion sites on the X chromosome of revertant line 91 has not been illustrated, but is identical to those of revertants 90 and 93.

whereas between these two strains many *copia* locations differed, reflecting the polymorphism present in the original v^{24} strain. Among the revertants of *D1*, three came from lines that had never been crossed with the *y v f mal* strain (lines 72, 81 and 101). Comparison with *D1* showed that *copia* was stable in these strains, even though they had been submitted to P-M hybrid dysgenesis. In striking contrast, 14 out of the 15 revertants obtained in the *y v f mal* genetic context showed changes in the sites of insertion of *copia* (the only exception is line 36).

Cloning of DNA sequences from the ovo locus

The insertion, in 12 revertants, of a *gypsy* element in the cytological region of *ovo* strongly suggests that reversion of the dominant *ovo*^{D} mutations is caused by this insertion. If this is indeed the case, it should be possible to clone DNA from the *ovo* locus by the method of transposon tagging (Bingham *et al.*, 1981). A genomic library was prepared with DNA from flies of revertant line 37, which contains only three gypsy euchromatic insertions. The library was screened with a full-length *gypsy* element. Forty positive clones were tested by *in situ* hybridization. Two clones, called G28 and G30, were found, the DNA of which hybridized at subdivision 4E on salivary gland chromosomes from *D1* and *Q259* flies, which have no *gypsy* at 4E. Restriction maps of these two clones are shown in Figure 4A. In order to identify the DNA fragments flanking the *gypsy* insertion, Southern blots of restricted DNA of the G28 and G30 clones were probed with a nick-translated *gypsy* probe. The 3.3 kb *SalI* fragment of G30 does not hybridize with *gypsy* and was used to start a chromosome walk within the *ovo* locus. Two different *Drosophila* libraries were screened: one from strain Oregon R and another one which we constructed from *Q259* flies, both strains being wild-type for the *ovo* function. We have thus cloned 32 kb of wild-type DNA around the *gypsy* insertion site (Figure 4C).

A distal boundary of the *ovo* locus was obtained from the breakpoint of the *Df(1)bi*^{D2} deletion. Southern blots comparing digested DNAs from heterozygous *Df(1)bi*^{D2}*/FM7c* flies and from homozygous *FM7c*, *D1* and *K1075* flies (the latter strain originates from the same parent as *D1* and is wild type for *ovo*) were probed with the nick-translated 3.3 kb fragment of phage G30. The patterns of restriction fragments obtained using *Eco*RI, *Pst*I (Figure 5) and *Bgl*II (data not shown) restriction enzymes are consistent with the proximal breakpoint of *Df(1)bi*^{D2} being located somewhere between −4 kb and −5 kb of the walk (Figure 4C). Locating the breakpoint of *Df(1)bi*^{D2} allowed us to orient the cloned DNA with respect to the centromere.

DNA analysis in ovo^{D} *revertants*

Gypsy or *copia* insertions induce reversion of dominant gain of function alleles (*D1* and *D3*) towards loss of function (*ovo*^{r}) alleles. It is therefore reasonable to assume that these insertions affect coding or regulatory regions of the *ovo* gene. Thirteen revertants (11 induced by insertion of *gypsy* and two by insertion of *copia*) were studied at the molecular level. Southern blots, comparing DNA from flies having *gypsy* or *copia* at 4E with those of the dominant alleles *D1*, *D2* and *D3* on the one hand, and of the wild-type allele *K1075* on the other hand, were probed with the nick-translated 3.3 kb fragment of G30. Whatever the restriction

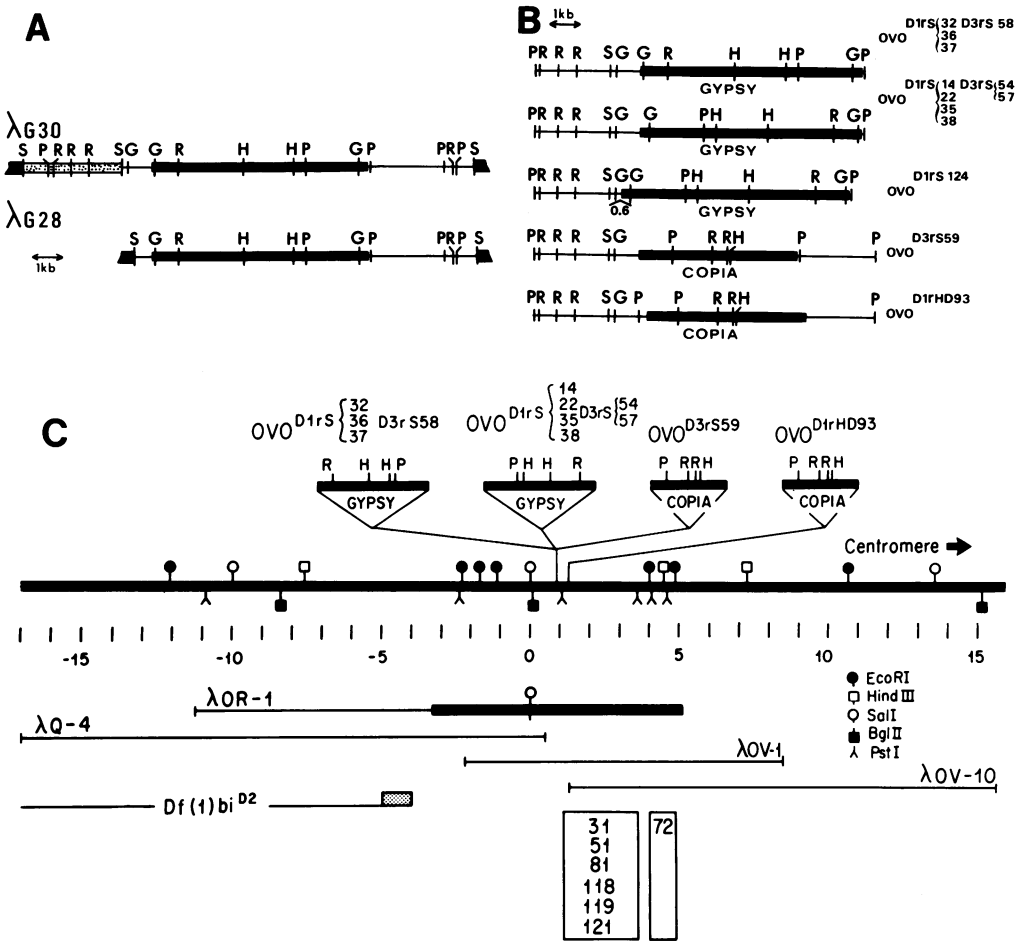

Fig. 4. (**A**) Restriction map of the *D.melanogaster* DNA segment in λG30 and λG28 hybrid phage clones. The solid bar indicates the *gypsy* element in the cloned segments and the stippled bar indicates the 3.3 kb *Sal*I fragment employed in subsequent analyses. (**B**) Restriction maps of DNA fragments from revertants containing *gypsy* or *copia* insertions. For revertants ovo^{D1rS37} and $ovo^{D1rHD93}$ the maps were deduced from digested DNAs of phages isolated from genomic libraries. For the other revertants, we considered the size of restricted fragments of genomic DNA. (**C**) Restriction map of the DNA region around a *gypsy* insertion at 4E. Sites of insertions of *gypsy* and *copia* are represented. These elements are drawn at a scale which is half the scale used to represent the genomic DNA. The EMBL3 λ clones derived from the region are represented by the horizontal lines below the restriction map. The solid bar on λOR-1 represents the 3.3 kb and 5.1 kb adjacent fragments, separated by a *Sal*I restriction site, used as probes in analyses. Deleted material in $Df(1)bi^{D2}$ is indicated by a line. The stippled box at the end indicates the uncertainty as to the location of its breakpoint. The locations of DNA rearrangements found in seven revertants of the dominant *D1* mutation are indicated by boxes. G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I.

enzymes used (*Eco*RI, *Pst*I, *Bgl*II or *Nco*I), restriction fragments of the three dominant alleles are indistinguishable from those obtained with the wild-type allele *K1075*. Revertants containing a *gypsy* insert show two different patterns of *Eco*RI fragments, corresponding to insertions of *gypsy* at the same site but in different orientations. In a first group, which includes revertant lines 32, 36 and 37, all issued from *D1*, and revertant 58, issued from *D3*, the 5.1 kb wild-type *Eco*RI fragment is lost and a new 3 kb fragment appears (Figure 6B, C). This result is consistent with all of these revertants having acquired a similarly oriented *gypsy* element at position +0.9 kb (see Figure 4B). In a second group, including revertants 14, 22, 35 and 38 and revertants 54 and 57, the 5.1 kb *Eco*RI fragment is replaced by an 8.8 kb fragment. This restriction pattern is consistent with the insertion of a *gypsy* element at the same position (+0.9 kb) as for revertants of the first group, but in the opposite orientation. This interpretation is confirmed by using *Pst*I, which cuts *gypsy* asymmetrically, as *Eco*RI, but near the opposite end (Figure 6D). Interestingly, all the *D1* revertants that bear *gypsy* in one orientation (14, 22, 35 and 38) are accompanied by a *lzl* phenotype; that phenotype is absent in *D1* revertants with *gypsy* in the opposite orientation, and in *D3* revertants. Revertant 124, which presents a *lzl* phenotype much stronger than the other *lzl* revertants studied here, appears to lack ~0.6 kb in the region between the *Sal*I site at position 0 and the *Pst*I site of *gypsy* (Figures 4B and 6A).

The size distribution of *Eco*RI and *Pst*I restriction fragments from revertant 59 is consistent (within the precision of the molecular analysis) with the *copia* insert of that revertant being at the same position as the *gypsy* insertion of the 10 revertants described above (Figures 4B, C and 6C). In order to characterize revertant 93, which also has a *copia* insert at 4E, a library of genomic DNA from *93/M5* flies was constructed, and one phage hybridizing with both the 3.3 kb fragment of G30 and a *copia* probe was isolated. Restriction mapping of this phage with *Eco*RI, *Sal*I and *Pst*I

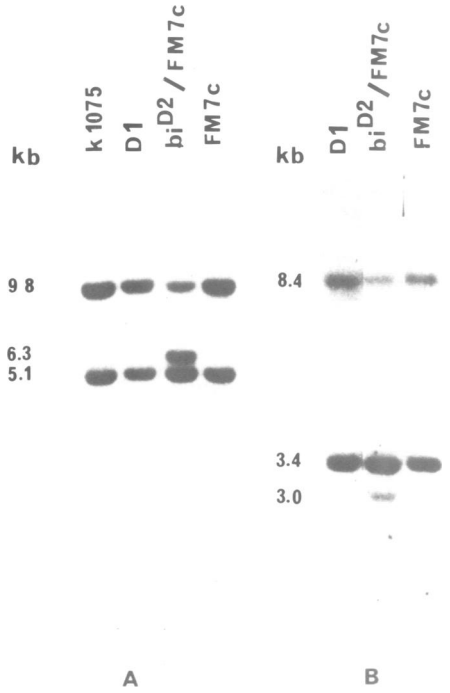

Fig. 5. Autoradiographs of Southern blots of restriction digested DNA. The probe is the 3.3 kb fragment (−3.3 to 0 kb in the walk) extracted after *Sal*I digestion from phage G30. (**A**) New *Eco*RI fragments generated by *Df(1)bi*^{D2}*/FM7c* flies in comparison with wild-type *K1075*, *D1* and homozygous *FM7c* flies. (**B**) *Pst*I restricted DNAs from *D1*, *Df(1)bi*^{D2}*/FM7c*, and homozygous *FM7c* flies.

showed that *copia* has inserted to the right and very close (~0.3 kb) to the *Pst*I site at position +1.1 kb (Figure 4B, C).

A number of revertants for which neither *gypsy* nor *copia* inserts were seen at 4E have also been examined. Among these were lines 81, 101 and 72, obtained from *P-M* dysgenic crosses (see Table II), as well as five other revertant lines, obtained in diverse genetic contexts, and all associated with the *svb* mutation. Southern blots of genomic DNA were probed with the above-mentioned 3.3 kb and 5.1 kb adjacent segments of DNA (see Figure 4C) which had been subcloned into the pUC13 plasmid vector. Although the 5.1 kb fragment appears to contain repeated sequences it could nevertheless be used in that type of analysis. No DNA alteration was observed in the three dominant alleles *D1*, *D2* and *D3* with the 5.1 kb probe. Probing Southern blots of restriction digests of genomic DNA from strain 72 with the 3.3 kb probe did not reveal any change with respect to the wild-type DNA (Figure 6B). However, the 5.1 kb probe revealed that, in this revertant, a DNA rearrangement, probably an inversion or insertion, has taken place within the 0.9 kb *Eco*RI segment which lies between +4 kb and +4.9 kb. In revertant 101 no DNA alteration was observed with either one of the 3.3 kb or 5.1 kb probes. Finally, *Eco*RI and *Pst*I digests of DNA from revertant line 81 are consistent with this revertant possessing an insertion of 5 kb within the *Pst*I DNA fragment that extends from positions +1.1 to +3.6 of the map (Figures 4C and 6B). The five additional revertant lines associated with the *svb* mutation that we have examined were balanced with *FM7c* (lines 31,

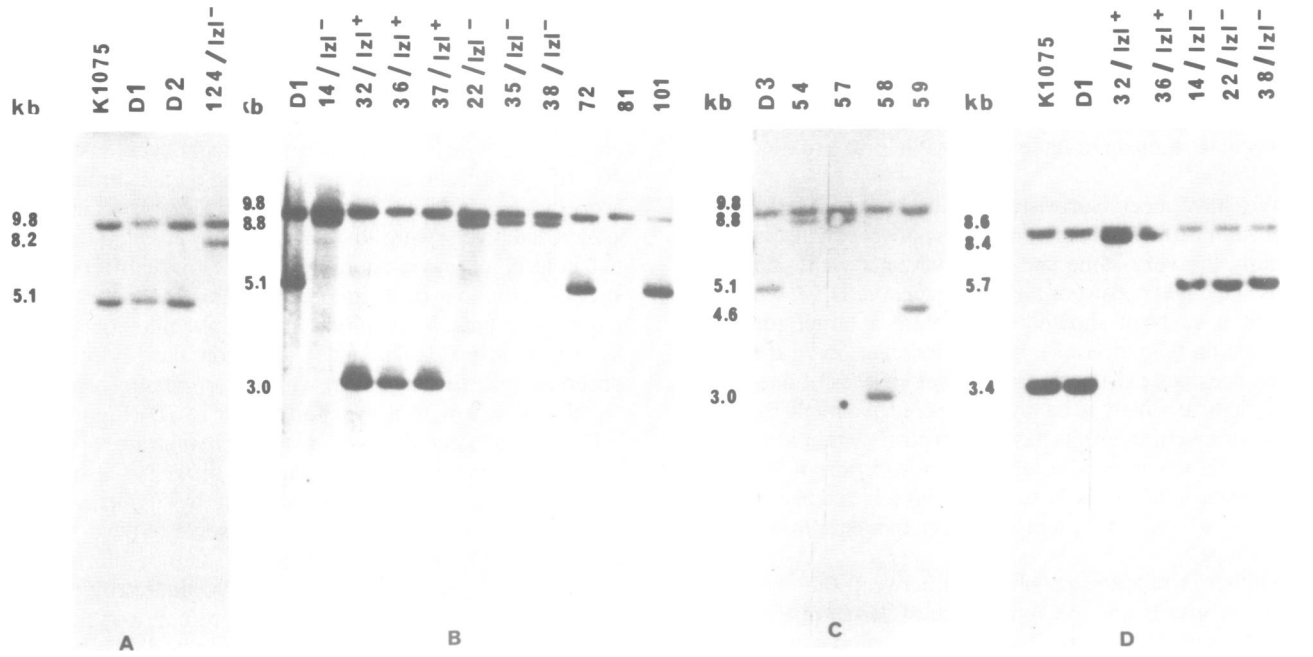

Fig. 6. Autoradiographs of Southern blots of restriction-digested DNAs from dominant and recessive *ovo* alleles. The probe is the 3.3 kb subcloned fragment (0 to −3.3 kb in the walk) extracted after *Sal*I digestion from phage G30. (**A**) *Eco*RI digest of DNA from wild-type *K1075* flies, *D1*, *D2* and the recessive revertant line 124 which contains a *gypsy* insertion and a 0.6 kb deletion. (**B**) Novel *Eco*RI fragments generated by *gypsy* insertion in either orientation in revertants of *D1*. Recessive *ovo*^{r} alleles associated or not with the *lzl* mutation are represented. Revertants 72, 81 and 101 were obtained from *P-M* dysgenic crosses. Revertants 72 and 101 do not exhibit changes in comparison with wild-type *K1075* flies. (**C**) *Eco*RI digest of DNAs from revertants of *D3* containing a *gypsy* insertion in either orientation (54, 57, 58), or a *copia* insertion (59). (**D**) Novel *Pst*I fragments generated by *gypsy* insertion in either orientation in revertants of *D1*.

51, 118, 119 and 121). Southern blots, comparing DNA from heterozygous flies with those of homozygous *FM7c* and *D1* flies, revealed novel restriction patterns. In each revertant a specific DNA rearrangement has occurred, but all of the rearrangements have taken place within the same *Pst*I segment of DNA (+1.1, +3.6) that was affected in revertant 81. The data suggest that an insertion could have taken place, the size of which is ~5 kb in the cases of revertants 31, 118, 119 and 121, and larger than 5 kb in the case of revertant 51.

Discussion

Delimitation of the ovo locus

Using *gypsy* DNA as a tag, we have cloned 32 kb of wild-type DNA around one *gypsy* insertion at 4E. Within this region we were able to locate the breakpoint of the deletion $Df(1)bi^{D2}$ which delimits distally the *ovo* locus. To establish that the region isolated indeed contains *ovo*, we compared DNAs of the three dominant ovo^{D} alleles with those of a variety of revertant alleles as well as of a wild-type allele. The region analysed covers 19 kb proximally to the breakpoint of $Df(1)bi^{D2}$. Almost all the revertants studied appear to result from insertions, not only of *gypsy*, but also of *copia* and of additional unidentified elements. These insertions are clustered into a small segment of DNA, no larger than 2.6 kb (revertant 72 stands alone in that it may extend proximally to 4 kb, the DNA section within which rearrangements are found). The three dominant ovo^{D} alleles, as well as revertant line 101, are indistinguishable from the wild-type strain, as far as restriction patterns of their genomic DNA are concerned. Since the dominant alleles were obtained by EMS mutagenesis, they could possibly result from point mutations within the DNA segment analysed. Alternatively, they could also consist of DNA rearrangements in more proximal regions that we have not yet analysed; further molecular studies are under way to delimit proximally the *ovo* locus.

We have been surprised to observe that, within the precision of the molecular analysis, all *gypsy* inserts at 4E occupy the very same site (10 revertants were analysed). This site could consist of one of the specific DNA sequences which have been shown to constitute a target for *gypsy* integration (Geyer *et al.*, 1986). However, even if a target sequence does exist at that site, other sources of integration specificity are likely to be involved. We have found that other types of insertion events have taken place within a restricted DNA region surrounding the site of insertion of *gypsy*; all these insertions are likely to have been selected because they delimit a region important for the sterile-dominant phenotype.

The strict relationship observed between the orientation of *gypsy* inserts and the appearance of the *lzl* phenotype in revertants of *D1* suggests that while insertion of *gypsy* does affect the *lzl* function, the site of insertion does not lie within the *lzl* structural gene. It is well known that the long terminal repeats (LTRs) of *gypsy* contain a variety of regulatory signals which can affect the expression of closely located genes (Freund and Meselson, 1984). Lower levels of transcripts relative to wild-type RNA have been reported for *y* and *f* mutations caused by *gypsy* insertions (Parkhurst and Corces, 1985; Geyer *et al.*, 1986). The opposite effect was observed in some *Hairy-wing* mutants in which *gypsy* increases the transcript levels by one order of magnitude (Campuzzano *et al.*, 1986). Depending upon the direction of transcription of the *gypsy* element inserted at 4E, transcriptional interference with the *lzl* gene could occur and cause the mutagenic effect.

No mutagenic effect resulting from integration of *gypsy* is observed on the neighbouring *svb* gene. In contrast, three insertions of *copia* and of other unidentified elements do induce the *svb* mutation in revertants. The *svb* and *ovo* functions have been genetically separated by the deletion $Df(1)bi^{D2}$ which fails to complement *svb* but retains ovo^{+} function. The insertions associated with the *svb* phenotype were all located into a 2.5 kb segment of DNA, well outside the portion of DNA deleted by $Df(1)bi^{D2}$. This result shows that DNA regions proximal to the breakpoint of $Df(1)bi^{D2}$ are important for the *svb* function. It is not clear why insertion of a *copia* element at position +1.3 kb of our walk does affect the *svb* function, while insertion of *copia* or *gypsy* in either orientation at position +0.9 kb does not (see Figure 4C). Depending upon whether the DNA sequences in which the insertions occur are coding, intronic or regulatory sequences, the *svb* function could be altered or unchanged. The results suggest that *ovo* and *svb* might be overlapping loci.

Mobilization of gypsy and other transposable elements in y v f mal × ovo^{D} crosses

The *y v f mal* strain appears to be endowed with particular properties since crossing it with ovo^{D} males results in high-frequency insertion of the *gypsy* element into the *ovo* locus. As was reported before (Busson *et al.*, 1983), these crosses result also in the appearance of X-linked mutations, some of which are lethal and others have visible phenotypes (up to now, only recessive X-linked mutations have been scored, but autosomal mutations are most likely also to occur). We have shown that flies of the *y v f mal* stock contain a variable and unusually high number of euchromatic *gypsy* insertions: 40–65 hybridization sites were counted on the chromosome arms, while in *D1* and *D3* only two and three *gypsy* insertion sites respectively were visible. Due to a possible differential control of *gypsy* mobility in the *y v f mal* and ovo^{D} strains, crossing these two strains might trigger *gypsy* mobility, eventually resulting in multiple mutations caused by *gypsy* inserts. The latter situation has indeed been observed in revertant 58, in which a *ct* mutation appeared simultaneously with a *gypsy* insertion at 7B (Figure 2B).

Crosses between the ovo^{D} and *y v f mal* strains appear to result in the mobilization not only of *gypsy* but also of the *copia* element. This was confirmed by comparing the distribution of *copia* on the X chromosome of the dominant *D1* and *D3* lines and of 18 revertants. Our results suggest that transposition of the *gypsy* and *copia* retrovirus-like elements may be under the same coordinate control. The question has been raised whether *P-M* hybrid dysgenesis is a stimulus for the movement of mobile elements other than *P*. Negative results (Woodruff, 1987) as well as positive ones (Rubin *et al.*, 1982; Gerasimova *et al.*, 1985) have been reported. In our own series of experiments, we did not observe that hybrid dysgenesis increases the frequency of reversion. However, it was very striking to note that all three revertants obtained in the course of a dysgenic cross between *y v f mal* females and ovo^{D} males possess a *copia* insertion rather than a *gypsy* one. This result suggests that *P-M* hybrid

dysgenesis could indeed stimulate the insertion of *copia* into new chromosomal sites.

To our knowledge it is the first time that a *Drosophila* strain is found that mobilizes the retrovirus-like elements *copia* and *gypsy*. The *ovo*^{D} mutations constitute a powerful tool for testing the capacity of any *Drosophila* strain to mobilize transposable elements. Every insertion event in an *ovo*^{D}/+ germ-line cell, provided that it occurs at the proper time of development, will result in female fertility and will be detected.

Materials and methods

Drosophila stocks

The dominant female-sterile mutations *Fs(1)ovo*^{D1} (= *Fs(1)K1237*), *Fs(1)ovo*^{D2} (= *Fs(1)K1103*) and *Fs(1)ovo*^{D3} (= *Fs(1)K155*) are marked with v^{24} and were kept in stocks as *ovo*^{D}/*Y* males by *C(1)DX,y f/Y* attached-X females. As wild-type homologs of *D1* we used the strain *K1075* (Komitopoulou *et al.*, 1983). Both *K1075* and *D1* are issued from the same v^{24} line which had been isogenized for the X chromosome. Dominant and recessive *ovo* mutations were maintained in conditions which exclude legitimate recombination in the X chromosome. Usually, *ovo*^{r} revertants associated with *svb* were balanced with *FM7c*. However, occasionally they were kept in stocks as *ovo*^{r}/*Y* males by attached-X females. This allowed us to collect male larvae for *in situ* hybridization as well as male flies for preparation of genomic DNA. Pure *ovo*^{r} revertants and those associated with *lzl* were maintained in *FM3* stocks. A *D1* strain with *P* cytotype, *D1(P)*, was constructed by crossing *D1* males with *C(1)DX,y f/Y* females presenting a *P* cytotype and then backcrossing for several generations. *Svb*^{YD39} was obtained from E.Wieschaus, *rg* from the Bowling Green Center, and the strain *Q259*, transformed with a *white* transposon, from D.Thierry-Mieg and G.Rubin. Visible mutations and balancers used are described in Lindsley and Grell (1968). Flies were grown on the standard Gif medium (Gans *et al.*, 1975) at 25 or 23°C.

Screening for revertants of *ovo*^{D}

Homozygous virgin *y v f mal* or *M5* females were mated to *ovo*^{D}/*Y* males. The resulting *ovo*^{D}/+ daughters were crossed with their brothers. In case of crosses implicating the *y v f mal* strain, sets of 20 females in tubes were observed; however, for crosses resulting in very low reversion events, females were distributed by 100 in bottles. Tubes or bottles with eggs and larvae were selected. *D1*/+ fertile females could be recognized by observing the ovaries through the cuticle of the abdomen, and afterwards isolated. *D3*/+ females presented nearly normal ovaries, and all females had to be isolated in order to find the fertile revertant. Revertants associated with *svb* could be detected by the deficit in emerging sons. In this case the line was established by crossing one *ovo*^{r}/+ daughter with *FM7c*/*Y* males.

In order to induce reversion using *P-M* hybrid dysgenesis, several breeding schemes have been used (see Table II). Homozygous *M5* females with *M* cytotype were crossed to *D1(P)* males. Alternatively, *M5* females with either *M* or *P* cytotype were mated with *D1(HD)* males issued from the dysgenic cross: *C(1)DX,y f/Y (M)* ♀♀ × *D1(P)* ♂♂.

We adopted for the names of revertants the same nomenclature as Oliver *et al.* (1987). *D1*rS = spontaneous revertant of *D1*; *D1*rHD = hybrid dysgenesis induced revertant of *D1*. In the text, for convenience, the revertants are referred to as numbers. The reversion of the *ovo*^{D} mutations towards recessive alleles was checked in each of the potential revertants by complementation with a loss of function allele, *ovo*^{r}, which had already been characterized.

In situ hybridization

To localize *gypsy* and *copia* on the chromosomes we used respectively the clones Dm111 (Bayev *et al.*, 1984) and cDm2087 (Dunsmuir *et al.*, 1980), both incorporated at the *Bam*HI site of pBR322. The 1.1-kb *Sal*I–*Xba*I fragment of the *white* gene (coordinates −0.7, +0.4 in the *white* restriction map of Levis *et al.*, 1982), was incorporated into the plasmid pSP64 and used as a probe to localize the site of insertion of the *white* transposon into the strain *Q259*. Salivary gland chromosomes were prepared according to Bonner and Pardue (1976). Labelled DNA was obtained by nick-translation with biotin-11-dUTP and hybridized to chromosome preparations in 0.6 M NaCl, 50 mM Na_3PO_4, 5 mM $MgCl_2$, 1 × Denhardt's solution for 12 h at 58°C. Development was performed as described in the Enzo Detek kit.

Preparation and screening of recombinant DNA libraries

Adult *Drosophila* genomic DNA was isolated as described by Bingham *et al.* (1981). Genomic DNA from wild-type *Q259* flies or from revertant lines 37 and 93 was partially digested with *Mbo*I and size fractionated on agarose gels. The 12–20 kb size range fragments were ligated into the *Bam*HI site of EMBL3 phage (Murray, 1983) and an *in vitro* packaging reaction was carried out. A library of wild-type Oregon R *D.melanogaster* DNA, inserted in the EMBL3 phage vector, was used to initiate a chromosomal walk. The screening of recombinant DNA libraries, phage and plasmid DNA preparation, DNA fragments isolation, ligations and subcloning of DNA into plasmid vectors were carried out as described in Maniatis *et al.* (1982).

Southern blot analysis

Restriction endonuclease-digested genomic DNA (5 μg) was fractionated on 0.8% agarose gels. DNA was transferred to nitrocellulose filters (Maniatis *et al.*, 1982) and Southern blots were hybridized with nick-translated probes in 5 × SSC, 50% formamide, 5 × Denhardt's solution and 50 μg/ml salmon sperm DNA at 42°C and washed at 42°C for 1 h in 1 × SSC, 0.1% SDS.

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References

Bayev,A.A.,Jr, Lyubomirskaya,N.V., Dzhumagaliev,E.B., Ananiev,E.V., Amiantova,I.G. and Ilyin,Y.V. (1984) *Nucleic Acids Res.*, **12**, 3707–3723.

Banga,S.S., Bloomquist,B.T., Brodberg,R.K., Pye,Q.N., Larrivee,D.C., Mason,J.M., Boyd,J.B. and Pak,W.L. (1986) *Chromosoma*, **93**, 341–346.

Bingham,P.M., Levis,R. and Rubin,G.M. (1981) *Cell*, **25**, 693–704.

Blackman,R.K., Grimaila,R., Koehler,M.M.D. and Gelbart,W.M. (1987) *Cell*, **49**, 497–505.

Bonner,J.J. and Pardue,M.L. (1976) *Chromosoma*, **58**, 87–99.

Bregliano,J.C. and Kidwell,M.G. (1983) In Shapiro,J.A. (ed.), *Mobile Genetic Elements*. Academic Press, New York, pp. 363–410.

Bucheton,A., Paro,R., Sang,H.M., Pelisson,A. and Finnegan,D.J. (1984) *Cell*, **38**, 153–163.

Busson,D., Gans,M., Komitopoulou,K. and Masson,M. (1983) *Genetics*, **105**, 309–325.

Campuzzano,S., Balcells,L., Villares,R., Carramolino,L., Garcia-Alonso,L. and Modolell,J. (1986) *Cell*, **44**, 303–312.

Dunsmuir,P., Brorein,W.J.,Jr, Simon,M.A. and Rubin,G.M. (1980) *Cell*, **21**, 575–579.

Freund,R. and Meselson,M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4462–4464.

Gans,M., Audit,C. and Masson,M. (1975) *Genetics*, **81**, 683–704.

Gerasimova,T.I., Matjunina,L.V., Mizrokhi,L.J. and Georgiev,G.P. (1985) *EMBO J.*, **4**, 3773–3779.

Geyer,P.K., Spana,C. and Corces,V.G. (1986) *EMBO J.*, **5**, 2657–2662.

Hazelrigg,T., Levis,R. and Rubin,G.M. (1984) *Cell*, **36**, 469–481.

Komitopoulou,K., Gans,M., Margaritis,L.H., Kafatos,F. and Masson,M. (1983) *Genetics*, **105**, 897–920.

Levis,R., Bingham,P.M. and Rubin,G.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 564–568.

Lindsley,D.L. and Grell,E.H. (1968) Carnegie Institute of Washington Publication, 627.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Modolell,J., Bender,W. and Meselson,M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1678–1682.

Murray,N. (1983) In *Phage Lambda and Molecular Cloning II*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 395–432.

Oliver,B., Perrimon,N. and Mahowald,A.P. (1987) *Genes Dev.*, **1**, 913–923.

Parkhurst,S.M. and Corces,V.G. (1985) *Cell*, **41**, 429–437.
Rubin,G.M., Kidwell,M.G. and Bingham,P.M. (1982) *Cell*, **29**, 987–994.
Rubin,G.M. (1983) In Shapiro,J.A. (ed.), *Mobile Genetic Elements*. Academic Press, New York, pp. 329–361.
Wieschaus,E., Nüsslein-Volhard,C. and Jürgens,G. (1984) *Wilhelm Roux's Arch. Dev. Biol.*, **193**, 296–307.
Woodruff,R.C., Blount,J.L. and Thompson,J.N.,Jr (1987) *Science*, **237**, 1206–1208.
Yannopoulos,G., Stamatis,N., Monastirioti,M., Hatzopoulos,P. and Louis,C. (1987) *Cell*, **49**, 487–495.

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