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P-lacW Insertional Mutagenesis on the Second Chromosome of *Drosophila melanogaster*: Isolation of Lethals With Different Overgrowth Phenotypes

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ABSTRACT

A single *P*-element insertional mutagenesis experiment was carried out for the second chromosome of *Drosophila melanogaster* using the *P-lacW* transposon. Out of 15,475 insertions on the second chromosome, 2,308 lethal and 403 semilethal mutants (altogether 2,711) were recovered. After eliminating clusters, 72% of the mutants represent independent insertions. Some of the mutants with larval, prepupal or pupal lethal phases have a prolonged larval period and show gradual overgrowth of the imaginal discs, brain and/or the hematopoietic organs (lymph glands). In this paper, 16 overgrowth mutants are described. As revealed by *in situ* hybridization, none of the mutations corresponds to any of the previously known overgrowth mutations on the second chromosome.

RECESSIVE lethal mutations causing hyperplastic or neoplastic overgrowth of the imaginal tissues in *Drosophila melanogaster* have been subjected to extensive studies recently (JACOB *et al.* 1987; BRYANT *et al.* 1988; LOFFLER *et al.* 1990; SZABAD, JURSNICH and BRYANT 1991; WOODS and BRYANT 1991). These types of mutations are particularly interesting from a developmental point of view. They are thought to interfere with the tightly controlled program of cell proliferation and differentiation by disrupting the functions of genes necessary for normal cell-to-cell communication. Morphogenesis of the imaginal discs is presumed to be coordinated through position-specific interactions between neighboring cells (BRYANT and SIMPSON 1984). The transmission of this positional information is thought to be disturbed in these mutants (MERZ *et al.* 1990; WOODS and BRYANT 1991).

Another interesting aspect of these mutants is that some of them show the basic features of tumorous growth (GATEFF 1978a) such as *in situ* overproliferation, altered cell morphology, inability to differentiate, invasiveness and transplantability. The genes of Drosophila that give rise to such mutant phenotypes are often called recessive oncogenes or tumor suppressor genes by analogy with their mammalian counterparts (GATEFF and MECHLER 1989). Overgrowth mutations therefore provide an excellent tool to investigate the processes underlying cell proliferation and differentiation in imaginal tissues (BRYANT and SCHMIDT 1990) and at the same time serve as a Drosophila model of tumorigenesis (GATEFF and MECHLER 1989). Characterization of these genes at the molecular level has also begun (MECHLER, MCGINNIS and GEHRING 1985; WOODS and BRYANT 1991).

Most of the known Drosophila overgrowth mutations are late larval or pupal lethals that affect the presumptive adult organs, while leaving the tissues necessary for larval survival mostly unaffected. The majority of such mutations display a prolonged larval period that can be explained by the presence of nondifferentiating growing imaginal rudiments preventing ecdysone release (SIMPSON, BERREUR and BERREUR-BONNENFANT 1980; POODRY and WOODS 1990). There have been several papers reporting the isolation of recessive late larval-pupal lethal mutations (SHEARN *et al.* 1971; STEWART, MURPHY and FRISTROM 1972; KISS *et al.* 1976; GATEFF 1978b; PERRIMON, ENGSTROM and MAHOWALD 1989) mainly on the *X* and the third chromosomes. Complementation data and statistical analysis of these mutants suggest that many of these genes have not been detected in the reported screens (SHEARN 1974).

In this paper we describe the results of a large-scale single *P*-element insertional mutagenesis experiment aimed at identifying most of the genes that can give rise to overgrowth-type mutations on the second chromosome of *Drosophila melanogaster*.

MATERIALS AND METHODS

Strains: *y w P-lacW* (BIER *et al.* 1989) was kindly provided by YUH NUNG JAN. The *P-lacW* insert contains the *P*-element transposase-*lacZ* fusion gene, the mini-*white* marker gene, a bacterial origin of replication, the β-lactamase gene and polylinkers facilitating cloning by plasmid rescue. *Sb* $P(ry^{+}\Delta 2\text{-}3)$—hereafter denoted as *Sb* $\Delta 2\text{-}3$—is the standard transposase source (ROBERTSON *et al.* 1988). Both strains were isogenized for the second chromosome before starting the experiment. *R(21)* means $Fs(2)Ketel^{r}R21$ (from M. ER-

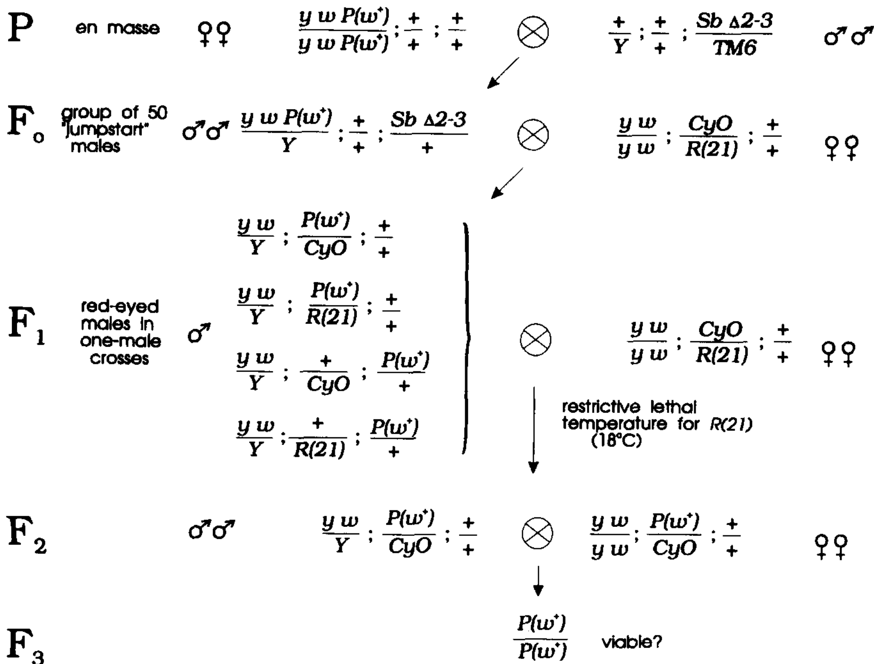

FIGURE 1.—Isolation scheme of second chromosome lethal insertions. $P(w^+)$ stands for the *P-lacW* transpozon carrying the w^+ marker gene. For details see text.

DÉLYI, unpublished data), a dominant cold-sensitive lethal revertant of $Fs(2)Ketel^1$ (SZABAD *et al.* 1989) that is a dominant female-sterile mutation. The y^+CyO balancer chromosome carrying a $P(y^+)$ insertion on the original *CyO* was kindly provided by ALLEN SHEARN. For a description of the genetic symbols and balancers used see LINDSLEY and ZIMM (1992). Both the *R(21)* and the y^+CyO chromosomes were introduced into a *y w* background. The flies were reared on standard yeast-cornmeal-agar medium and all experiments were performed at 25° except otherwise noted.

***P*-element mutagenesis:** The mutagenesis scheme is shown in Figure 1. Females homozygous for the *P-lacW* insertion on the *X* chromosome were crossed to males carrying *Sb Δ2-3* on the third chromosome over the *TM6* balancer. This cross yields F_0 "jumpstart" males carrying both the *P-lacW* and the *Δ2-3* elements together, in which the *P-lacW* gets mobilized and "jumps" into new insertion sites. F_0 "jumpstart" males were crossed to *y w/y w; R(21)/CyO* females in groups of 50 males and 100 females. The dominant cold-sensitive lethal *R(21)* was used to eliminate the unwanted progeny in the subsequent generations. F_1 males having an insertion on the autosomes were identified by their colored eyes due to the mini-*white* marker gene. Such males not carrying the *Sb Δ2-3* chromosome were crossed individually to *y w/y w; R(21)/CyO* females. After 3 days, these one-male crosses were transferred to the restrictive temperature of 18° where the progeny classes carrying *R(21)* were killed. Thus, all the second chromosome insertions resulted in balanced F_2 stocks. Insertions on other autosomes were recognized by the appearance of white-eyed individuals in F_2 and were discarded. The balanced second chromosome stocks were tested for lethality in the next generation. The nonlethal lines having Cy^+ flies among the progeny were also discarded.

Lethal phase analysis: To analyze the lethal phase, the *CyO* balancer was replaced by the y^+CyO chromosome. As the mutant stocks were homozygous for *y w*, lethal larvae carrying the *P-lacW* insertion on both second chromosomes were easily identified according to their yellow mouth hooks while the balancer-carrying heterozygotes had wild-type (black) mouthparts. Eggs were collected from each line for 1 day in bottles and the larvae were washed out of the medium just before pupariation, 6 days after egg laying (AEL). Larval viability was examined by estimating the ratio of homozygous mutant *yellow* larvae to the y^+ ones upon washing out. The homozygous mutant larvae were transferred to fresh food in vials and kept in a humidified atmosphere at 25°. The larvae were checked every 3–4 days until all animals died as larvae or at further stages of development. If no *yellow* larvae were found at washing out, the mutation was tentatively classified as embryonic lethal. For the above reason, the "embryonic" lethal class probably also included those mutants that hatched but died as young larvae. In the "early larval" lethal category (Table 1) small underdeveloped *y* larvae were found upon washing out. These usually survived during the prolonged incubation period, but failed to grow and eventually died.

TABLE 1

Lethal phases of the *PlacW* insertion mutants

Effective lethal phase	Number of isolates	%
Embryonic	1265	46.7
Embryonic-larval	105	3.9
Early larval	248	9.1
Late larval	**57**	**2.1**
Prepupal	**40**	**1.5**
Pupal	**220**	**8.1**
Pharate adult	252	9.3
Adult (semi-lethal)	403	14.9
Polyphasic lethal	121	4.1
Σ:	2711	100.0

Further lethal classes were distinguished according to the developmental phase where the lethal arrest mostly occurred. Altogether, 30–50 *yellow* larvae were analyzed for each mutant line.

Analysis of the lethal phenotypes: As the overgrowth phenotype in general develops gradually during a prolonged larval period, 280 lines of the late larval, prepupal and pupal lethal categories showing a prolonged larval period were

subjected to a more detailed phenotypic analysis. On day 6 AEL, 100 homozygous *y* larvae were selected and kept in fresh vials under humid conditions as above. At 2–3 day intervals, 5–10 larvae were dissected and the internal organs examined under a stereomicroscope. Special attention was paid to the imaginal discs, the brain and the hematopoietic organs (lymph glands) because these tissues show abnormal growth in other overgrowth mutations.

Isolation of viable revertants: To ascertain whether the lethality was caused by the *P-lacW* insertion, the *P* element was remobilized to induce precise excisions. Females from the mutant strains were crossed to *Sb* Δ*2-3/TM6* males (see Figure 2 for the detailed scheme). "Jumpstart" males from the progeny were backcrossed to females of the same mutant strain. Progeny from the latter cross were screened for the appearance of viable Cy^+ individuals carrying revertant chromosomes derived from precise excision of the *P-lacW* insert. Cy^+ revertant males were crossed to Sco/y^+CyO females to establish balanced revertant stocks. These stocks were tested for loss of the *P-lacW* insertion by *in situ* hybridization.

Cluster analysis and complementation: Premeiotic insertion of the *P* element leads to appearance of the same mutation in multiple progeny forming a mutant cluster. Since in the mutagenesis experiment the F_0 jumpstart males were crossed in groups of 50 (Figure 1) and numerous inserts were recovered from each group, the appearance of clusters was inevitable. To assist in the identification of the clusters, we marked every mutant line with a double number; the first or main number represented the group of 50 F_0 ancestor males, the second represented the serial identification number within a given F_0 group. By definition, members of a cluster show the same phenotype. To identify the clusters, complementation crosses were performed between lines of the same main number having the same lethal phase.

Complementation analysis within the mutants with overgrowth phenotypes was also performed to determine whether independent alleles of the same gene had been isolated.

***In situ* hybridization:** Chromosome squashes were made as described by ATHERTON and GALL (1972). *P-lacW* insertions were detected using digoxigenin-labeled probes prepared with the DIG DNA labeling and detection kit (Boehringer).

RESULTS

Isolation of *P*-insertion mutants: A large-scale single *P*-element mutagenesis was performed using the *P-lacW* construct, aiming to saturate the second chromosome with recessive lethal insertions. The relatively high transposition frequency of *P-lacW* facilitated the recovery of such mutations.

With F_1 males representing single autosomal insertions (Figure 1), 44,452 one-male crosses were established of which 31,384 proved to be fertile. They were distributed between the second chromosome and the other autosomes almost equally; 15,475 and 15,909 insertion lines, respectively. Of the 15,475 *P* insertions in the second chromosome, 2,308 proved to be lethal and 403 semilethal—altogether 2,711 (17.5%)—while the rest (82.5%) remained homozygous viable.

Lethal phases of the mutants: The insertion mutants are classified according to their lethal phases in Table 1. The proportion of the embryonic lethal category, comprising 46.7% of all the lethal isolates, is higher than obtained in previous mutagenesis experiments (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; PERRIMON, ENGSTROM and MAHOWALD 1989). This can be explained by the embryonic lethal category also including some of the early larval lethals as was noted in MATERIALS AND METHODS. Accordingly, the early larval lethal category is underrepresented in our data (9.1%). The proportion of mutants in the pharate adult and semilethal category is also remarkably high (9.3% and 14.9%, respectively). Many *P*-element insertions may cause weak hypomorphic mutations that fall into the semilethal or pharate adult categories leading to their high proportion. The late-larval, prepupal and pupal lethal categories represented by 317 isolates (11.7%) are the most interesting ones with respect to overgrowth, so they were further characterized.

Lethal phenotypes: Mutations interfering with normal development of the imaginal tissues frequently cause larval or prepupal-pupal lethality. Most of the known overgrowth mutants fall into these lethal categories (BRYANT and SCHMIDT 1990), so our search was concentrated on the larval-pupal lethals. We preferentially examined those mutant stocks in which the homozygous larvae had good viability and remained in the larval stage for a substantial time after the pupariation of their normal sibs. The internal organs of 280 such lines belonging to the late larval, prepupal and pupal lethal categories were examined by dissection. One third of the dissected lines did not show any visible abnormalities. However, in the rest of the lines, the brain and imaginal discs were underdeveloped at the time of normal pupariation and reached the normal size or grew further only later. Lines in which the discs remained tiny during the prolonged larval development or no discs could be found at all were also observed. In many lines the imaginal discs displayed abnormal folding and the brain also acquired an abnormal shape. In some cases these abnormalities were slight, and their penetrance and expressivity were rather variable. This was also true for some of the overgrowth mutants.

Table 2 includes data regarding the 16 overgrowth mutants that consistently show the abnormal phenotype. According to the overgrown organ three basic phenotypic groups can be distinguished: the lymph gland overgrowth (n = 7), the disc overgrowth (n = 4) and the brain overgrowth (n = 1) mutants. In four other mutants these phenotypic traits appear in combination: both the lymph gland and the brain are affected in one mutant, lymph gland and discs in two mutants and discs and brain in one mutant.

Lymph gland overgrowth phenotype: These mutants

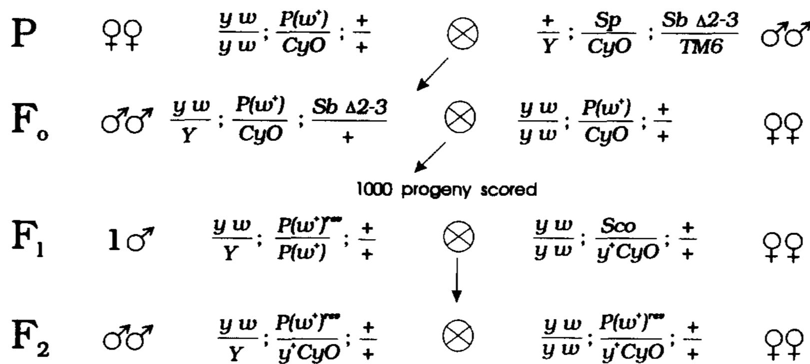

FIGURE 2.—Isolation of viable revertants by remobilizing the *P-lacW*. $P(w^+)$ stands for the PlacW transpozon. $P(w^+)^{rev}$ represents a revertant chromosome with precise excision of the *PlacW*. See text for details.

display extreme hypertrophy of the lymph gland lobes along the dorsal vessel. Two types of lymph gland defects can be distinguished in this set of mutants:

(i) In lines *l(2)144/1*, *l(2)30/7* and *l(2)88/10*, the hypertrophy is so extreme that the lymph gland frequently forms a structureless mass that can fill the anterior body cavity. Lymph gland fragments become detached, dispersed all over the body and often become melanized (Figure 3).

(ii) In lines *l(2)90/37*, *l(2)131/7*, *l(2)154/1*, *l(2)168/14*, *l(2)86/34*, *l(2)65/24* and *l(2)211/5*, the hypertrophic lymph gland retains its lobular structure and the overgrowth is not as extreme as in the previous category. The formation of melanotic capsules is infrequent. In both categories of lymph gland mutants the enlargement of the lymph gland is frequently accompanied by underdevelopment of the brain and imaginal discs.

In addition to lymph gland enlargement, lines *l(2)88/10* and *l(2)131/7* display disc overgrowth, while line *l(2)90/37* shows brain overgrowth.

The lymph gland overgrowth mutants listed in Table 2 can alternatively be classified in the "Class 2 *aberrant immune response*" (*air*) category according to WATSON, JOHNSON and DENELL (1991). In Class 2 *air* mutants the hematopoietic organs are enlarged and an aberrant immune reaction may be responsible for melanotic tumor formation. In Class 1 *air* mutants the lymph glands remain normal, and abnormal melanization is apparently a result of an autoimmune response against abnormal target tissues. We also isolated mutants with the Class 1 *air* phenotype, but they are not discussed in the present paper.

Imaginal disc overgrowth phenotype: In Table 2, seven mutants exhibit overgrowth of the imaginal discs. Six of them (*l(2)131/7*, *l(2)161/28*, *l(2)106/22*, *l(2)43/1*, *l(2)79/18* and *l(2)115/12*) belong to the hyperplastic overgrowth category according to BRYANT and SCHMIDT (1990), retaining the single layered epithelial structure of the imaginal discs. In the majority of the hyperplastic overgrowth mutants (*l(2)131/7*, *l(2)161/28*, *l(2)43/1*, *l(2)79/18* and *l(2)115/12*), the discs remain morphologically well defined but show abnormal folding and enlargement (Figure 4). In contrast, in line *l(2)106/22* the abnormally folded epithelia of the eye-antenna and labial discs seem to grow indefinitely, forming an irregular mass in the head (Figure 4E). All the hyperplastic disc overgrowth mutants are able to pupariate; lines *l(2)79/18* and *l(2)161/28* can even reach the pharate adult stage with many of the adult cuticular structures being abnormal.

Only line *l(2)88/10* belongs to the neoplastic overgrowth category since its disc epithelia show no folds and form spongy masses. These structureless discs are tiny, even as late as 6 days AEL beyond the normal time of pupariation. They grow gradually, exceed normal size and fuse with each other by the end of the prolonged larval period. The homozygotes die as larvae without pupariating.

Generally the discs do not overgrow to the same extent: usually the leg and eye-antenna discs are larger than the others (Table 2).

Strains *l(2)90/37* and *l(2)115/12* exhibit brain overgrowth in addition to disc enlargement. Lines *l(2)43/1* and *l(2)79/18*, which belong to the disc overgrowth group, also show occasional overgrowth of the brain.

Brain overgrowth phenotype: The brain overgrowth group comprises the above mentioned lines *l(2)90/37* and *l(2)115/12*, as well as *l(2)82/25*. In these mutants the optic lobes are enlarged and show protrusions resulting in an elongated or cuboidal shape of the hemispheres, while the ventral ganglion remains normal (Figure 5). The overgrown hemispheres are frequently asymmetric, as in line *l(2)115/12* where one hemisphere of the brain characteristically grows larger than the other.

Cluster analysis: To reveal possible clusters of identical mutants, complementation crosses were carried out among the lines that were derived from the same group of 50 F_0 males and had identical lethal phases. Of the 2711 lethal isolates, altogether 2399 were tested in this way. Table 3 summarizes the distribution of clusters according to their size. As the F_0 crosses were propagated for 10 days, we obtained essentially the entire progeny produced during the life span of

TABLE 2

Genetic and developmental characteristics of the overgrowth mutants

Mutant stock	Overgrown organs	Lethal phase	Life-span of larvae[a]	Penetrance[b]	Chromosomal location[c]	Remarks[d]
l(2)90/37	Brain and lymph gland	*P*	14–18	Brain: 50% (13 days) l.g.: 20% (15 days)	48E	Elongated br. hemispheres; slightly abnormal folding in discs; l.g. retains lobular structure
l(2)131/7	Lymph gland and wing discs	*P*	9–18	Discs: 28% (12 days) l.g.: 17% (13 days)	48E (49B)	Br. has normal size, abnormal shape; wing and haltere discs rarely showing outgrowth, folding abnormal; l.g. retains lobular structure
l(2)154/1	Lymph gland	*L-pP*	14–21	60% (12 days)	48E	Br. < n with abnormal shape; d. < n, folding abnormal; both d. and br. can exceed normal size at the end of larval life; l.g. retains lobular structure
l(2)168/14	Lymph gland	*P-(fA)*	13–19	56% (14 days)	23B	Br. normal in size, abnormal in shape; d. < n, with abnormal folding; both br. and discs grow with age; l.g. retains lobular structure
l(2)86/34	Lymph gland	*L*	11–20	35% (11 days)	37D	Br. < n; discs < n; l.g. retain lobular structure; melanotic stripes appear in larval cuticle before death; infrequent melanotic masses in larvae
l(2)65/24	Lymph gland	*L*	19–23	20% (12 days)	48A	Brain < n; discs < n; lymph gland retains lobular structure
l(2)211/5	Lymph gland	*L*	13–21	100% (12 days)	51	Brain < n; discs ≪ n; lymph gland retains lobular structure
l(2)144/1	Lymph gland	*L*	11–25	100% (16 days)	31A	Br. < n, d. < n with abnormal folding; l.g. grow as diffuse masses; melanotic masses are dispersed all over the body (typical *air* phenotype)
l(2)30/7	Lymph gland	*L-P*	24–29	20% (16 days)	55DE (23AB)	Brain < n; discs < n; l.g. grow as diffuse masses by the end of larval life
l(2)88/10	Lymph gland and discs	*L*	14–20	l.g.: 62% Discs: 100% (9 days)	56A	Br. < n, with elongated hemispheres; discs form structureless masses, sometimes fusing with each other and the brain, the eye-antenna discs are larger than the others; l.g. grow as diffuse masses; infrequent melanotic masses in larvae
l(2)161/28	Discs	*(P)-fA*	10–17	100% (8 days)	43B	Uniformly overgrown discs with abnormal folding
l(2)106/22	Eye-antenna discs	*P*	8–16	45% (13 days)	57	The antenna part is especially large, the eye part and the labial discs > n, other discs remain n
l(2)43/1	Discs	*P*	7–11	Discs: 100% (10 days)	38CD	Lymph gland = n; brain occasionally shows outgrowth; leg discs are especially overgrown; folding of all discs are abnormal from day 4
l(2)79/18	Discs	*P-(fA)*	11–17	77% (10 days)	22C	Brain occasionally > n; lymph gland = n; discs, (especially the legs) develop extra foldings; expressivity is much better in older larvae
l(2)115/12	Brain and discs	*P*	13–16	Br.: 50% (12 days) d.: 70% (9 days)	50E	Br. asymmetric, with elongated or cuboidal hemispheres; the leg discs especially large, folding normal
l(2)82/25	Brain	*L*	10–15	85% (13 days)	35DE	d. = n; l.g. = n, brain hemispheres become elongated from day 7

Abbreviations: br.: brain, d.: discs, l.g.: lymph gland, n: normal in size, <n or ≪n: smaller or much smaller than normal, >n: larger than normal.

[a] Age of larvae dying first and last, in days from egg collection (AEL). In case of pupal lethals, age of larvae pupariating first and last. (Control population pupariated on the 6th day AEL.)

[b] Penetrance of lethality = 100%. Penetrance of overgrowth phenotype = $\frac{\text{No. of larvae with overgrowth phenotype}}{\text{Total number of larvae dissected}} \times 100$. Numbers in brackets indicate the minimum time in days after egg laying required to see the phenotype. Penetrance was calculated after this time only.

[c] Cytological positions in brackets indicate second *P* insert not responsible for the mutant phenotype.

[d] Organs not noted are apparently normal.

the F_0 generation. The F_0 group produced an average of 23 (maximum 58) lethal F_1 isolates, so this was the maximum size of a potential cluster. The largest cluster turned out to consist of 16 members, but most of the isolates did not form clusters (Table 3). Altogether, 72% of all the inserts were independent and only 28% represented related events. Thus, the 2399 tested lethals represent 1748 independent *P*-element insertions. We did not test 312 lines for clusters because they belonged to F_0 groups with exceptionally large numbers of lethal isolates, requiring an excessive number of crosses.

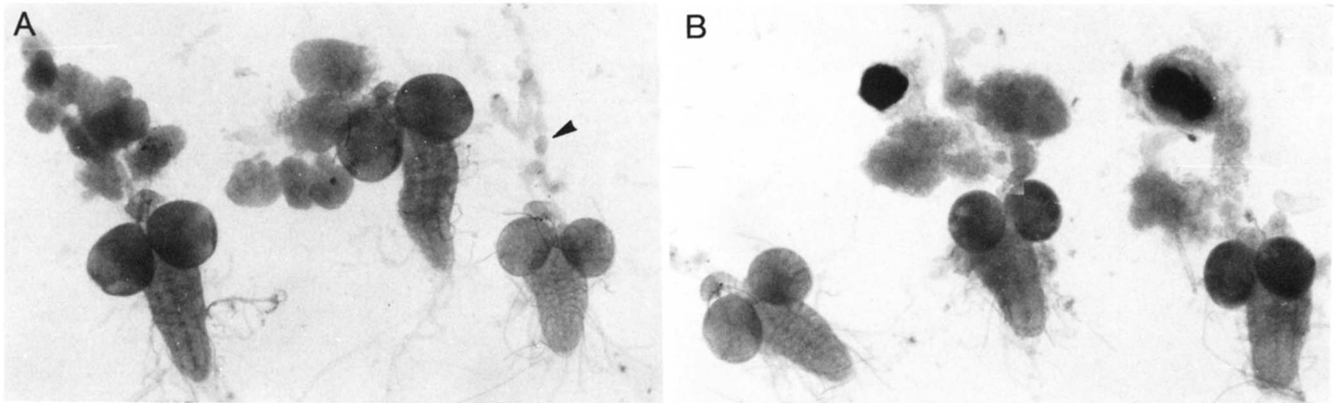

FIGURE 3.—Overgrown lymph glands of (A) *l(2)168/14* 18 days AEL and (B) *l(2)144/1* 20 days AEL. Arrow indicates normal lymph gland of a wild-type larva 5 days AEL. Notice the black melanotic capsules in the lymph glands of *l(2)144/1* (B).

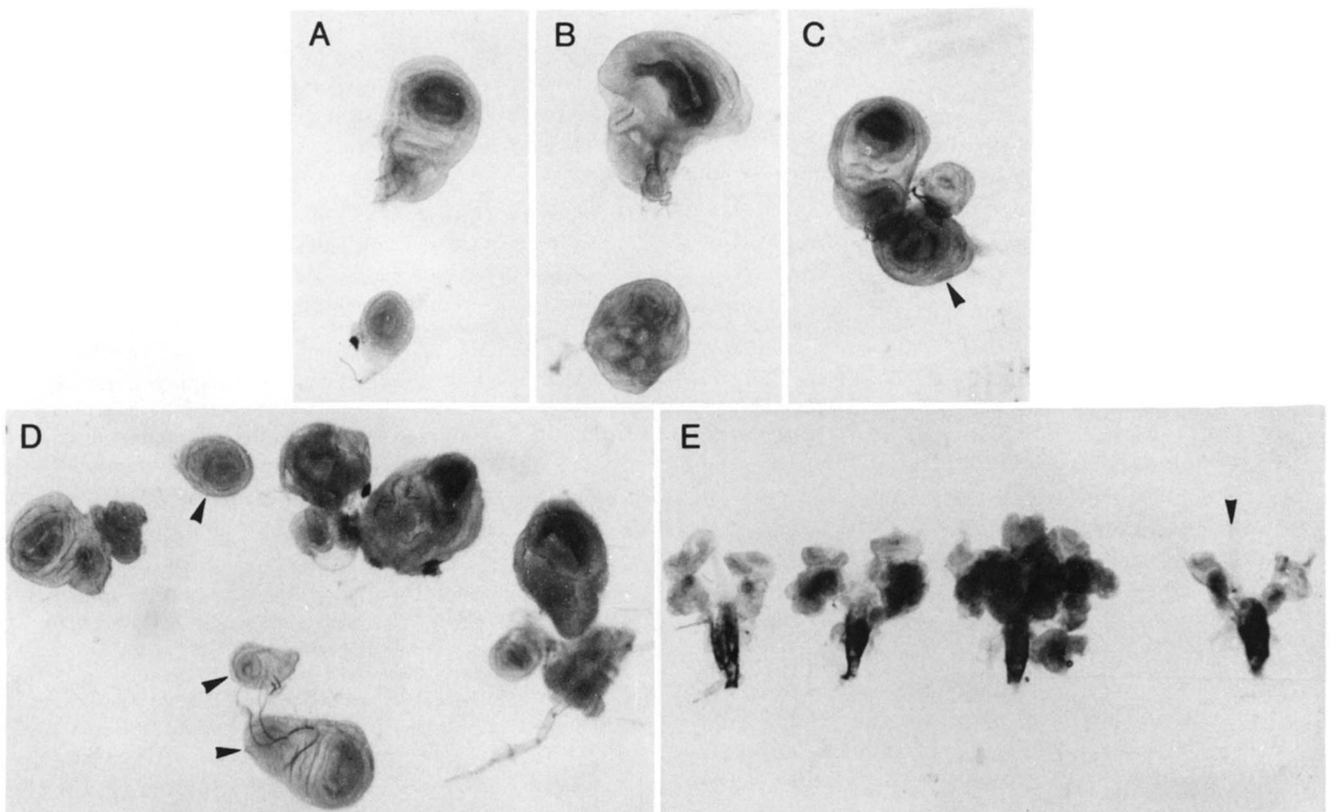

FIGURE 4.—Photographs of imaginal discs from disc overgrowth mutants. (A) Wing (top) and third leg (bottom) discs from a wild-type third-instar larva 5 days AEL. (B) Abnormal wing (top) and third leg (bottom) discs from *l(2)79/18* 15 days AEL. (C) Wing, haltere and third leg discs from *l(2)43/1* 10 days AEL. Arrow indicates the enlarged third leg disc (compare with wild type in Figure 4A). (D) Abnormal wing, haltere and third leg discs from *l(2)161/28* 15 days AEL. Arrows indicate control wing, haltere and third leg discs from wild-type third instar larva 5 days AEL. (E) Abnormal eye-antenna discs from *l(2)106/22*. From left to right: 12 days AEL, 14 days AEL and 16 days AEL. Arrow indicates control eye-antenna discs of a wild-type larva 5 days AEL.

Complementation: One case of true allelism was observed among the overgrowth mutants: *l(2)90/37*, *l(2)131/7* and *l(2)154/1*, which were derived from three different F_0 male groups and were localized to 48E by *in situ* hybridization, did not complement and therefore represent independent insertion events in the same locus. It is interesting that although the phenotypes of these alleles are similar to one another, they do show some differences (see Table 2).

Two other overgrowth mutants (*l(2)30/7* and *l(2)168/14*) both having insertions in the 23AB region did complement and therefore represent independent loci.

***In situ* hybridization:** The locations of the insertions as determined by *in situ* hybridization to polytene chromosomes are shown in Table 2. Based on chromosomal positions, none of our isolates corresponds to previously known overgrowth/tumorous mutations on the second chromosome (see GATEFF and MECHLER 1989 for review). While 14 of the examined mutants

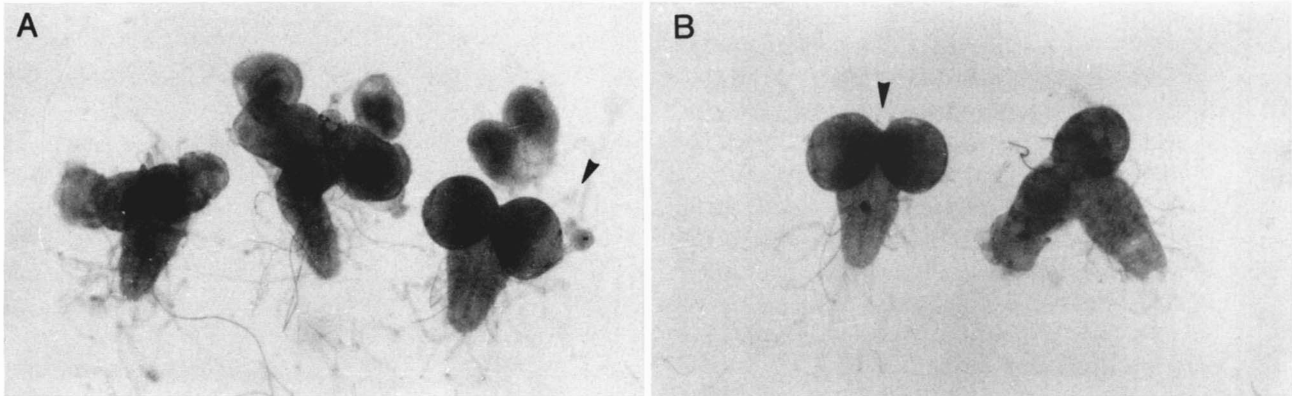

FIGURE 5.—(A) Overgrown brain hemispheres from *l(2)115/12* 15 days AEL. (B) Outgrowth on the left hemisphere of *l(2)43/1* 11 days AEL. Arrows indicate brains from wild-type larvae 5 days AEL.

TABLE 3

Distribution of clusters

Cluster size (no. of copies/locus) (A)	Number of independent clusters (B)	Number of lines (A × B)	
1 (no cluster)	1429	1429	59.5%
2	181	362	15.1%
3	59	177	7.4%
4	30	120	5.0%
5	23	115	4.8%
6	9	54	2.3%
7	10	70	2.9%
8	2	16	0.7%
9	3	27	1.1%
13	1	13	0.5%
16	1	16	0.7%
Σ:	1748	2399	100%
Not tested		312	
Total:		2711	

had single *P-lacW* insertions, two (*l(2)30/7* and *l(2)131/7*) had double inserts on the second chromosome. The possibility of more than one insertion in the genome was also supported by the observation that in some of the lethal lines (not listed in Table 2) the flies had different shades of the reddish eye color. In these mutant lines, two or three different shades of eye coloration were observed in flies of the same sex, irrespective of their age. Genetic crosses revealed that this phenomenon was caused by the presence of a balanced *P-lacW* insert in the second chromosome and an independently segregating one in another autosome.

Reversion of the *P*-induced mutations: In each of the 16 overgrowth stocks, the *P* element was remobilized and viable revertants were isolated. The absence of *P-lacW* from the former insertion sites in the revertants was also verified by *in situ* hybridization. This is a direct demonstration that the lethal phenotype is due to the insertion. The isolation scheme described in Figure 2 allows the recovery of precise excisions only. The frequency of reversion varied slightly from strain to strain, ranging from one to four viable revertant F_1 males per 1000 progeny.

Two other mutant lines also showing overgrowth phenotypes did not yield viable revertants, so they were not included in Table 2. These two nonreverting lines may contain second-site lethal mutations that prevent the appearance of viable revertants. Alternatively, the inserted *P* element may be defective and unable to remobilize. Experiments to test these possibilities are under way.

l(2)131/7 contains two *P* elements inserted in 48E and 49B. In its viable revertants the insert is missing from 48E while the other *P* remains in 49B (Figure 6). Therefore, the lethal phenotype is due to the 48E insertion and the other *P* in 49B is nonlethal. This is also true for line *l(2)30/7* where only one *P-lacW* in 55DE causes the lethality.

Chromosomal rearrangements: We do not know the frequency of gross chromosomal rearrangements among our mutants, but the fact that a pericentric inversion (*l(2)96/19*, not listed in Table 2) was found shows that they do exist in our collection. It is interesting that in spite of the rearrangement this stock readily gave viable revertants that lost the *P* insert while retaining the pericentric inversion. This suggests that the inversion breakpoints themselves must be homozygous viable.

The two mutants (*l(2)169/4* and *l(2)170/39*), which did not yield viable revertants, did not show any visible rearrangements.

DISCUSSION

Single *P*-element insertional mutagenesis has many advantages over traditional mutagenesis procedures: engineered *P*-element constructs like *P-lacW* allow direct cloning of the flanking sequences via plasmid rescue; the mutated locus can be easily localized by *in situ* hybridization; remobilizing the inserted element can generate new alleles; and expression patterns can

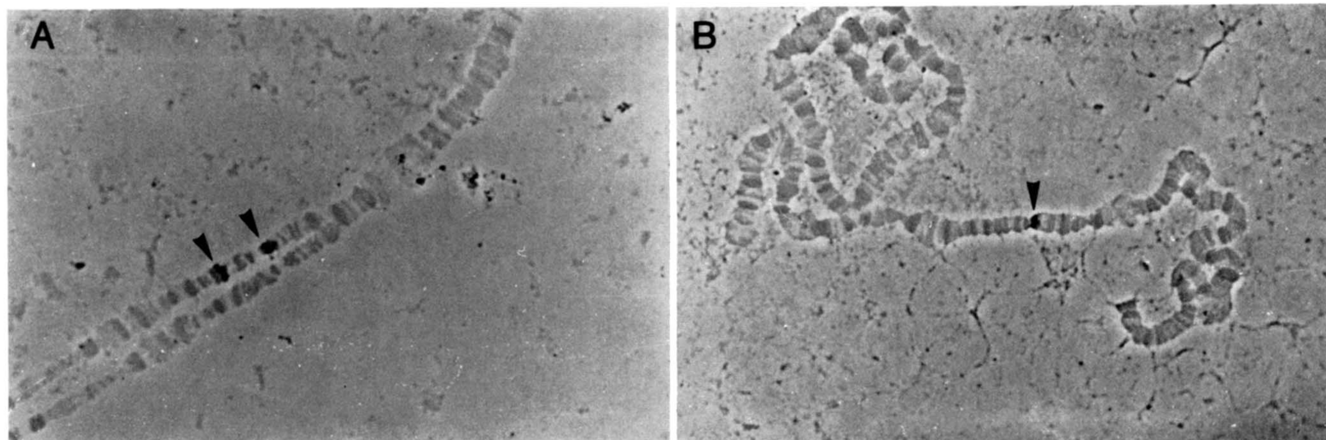

FIGURE 6.—Localization of the *P-lacW* insert by *in situ* hybridization on the giant chromosomes of (A) *l(2)131/7* and (B) *l(2)131/7^{rev}* (revertant) using DIG-labeled *PlacW* probe. Arrows indicate the inserts (A) in 48E and 49B, (B) in 49B. The insert in 48E is responsible for the mutant phenotype.

be characterized by *lacZ* staining of tissues. Nevertheless, the practical application of single *P*-element mutagenesis on a large scale was limited until recently due to the low mobilization frequency of the constructs. In the first large-scale experiment of COOLEY, KELLEY and SPRADLING (1988), approximately 12% of the "jumpstart" males produced at least one progeny having a transposition of the *pUChsneo* element from the *X* to the autosomes. Development of the enhancer detection technique led to the creation of a series of Drosophila lines carrying versatile *P*-element constructs (O'KANE and GEHRING 1987; GHYSEN and O'KANE 1989; BELLEN *et al.* 1989; WILSON *et al.* 1989; BIER *et al.* 1989). One of them—*P-lacW*—proved to have a remarkably high mobility when crossed to the generally used Δ*2-3* transposase source. The *P-lacW* transposes from the *X* to the autosomes in all (100%) of the jumpstart males in comparison with the 12% mobility of the *pUChsneo*. By making use of this high jumping frequency, we carried out a large-scale single *P*-element insertional mutagenesis of the second chromosome. We recovered 15,475 insertions, of which 2,711 (17.5%) were lethal or semilethal in homozygous condition. After eliminating the clusters of identical mutants, 2,399 lines represent 1,748 independent lethal insertions.

Level of saturation: The Drosophila genome is estimated to contain around 5000 lethally mutable genes (HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972). As the second chromosome comprises about two-fifths of the Drosophila genome, it must harbor roughly 2000 lethal loci. Assuming that the average integration frequency of *P-lacW* is the same for all the genes and the Poisson distribution fits, the 1748 independent lethals we recovered would mean a 58% saturation ($m = 1748/2000 = 0.874$ and $P(0) = m^0 \times e^{-m} = e^{-0.874} = 0.42$). However, it is generally accepted that the frequency of *P*-element integration varies from locus to locus, and it has also been suggested that almost half of the Drosophila genes are inaccessible to *P*-element insertions (KIDWELL 1986). It is likely that several integration "hot spots" attract the majority of the inserts while the rest are distributed among many low-frequency sites, and that there are loci that remain inaccessible for the *P* element. Thus, the 58% saturation based on the Poisson distribution is certainly a distorted value.

In a recent experiment (P. DEÁK, unpublished data) all the 2711 *P*-induced lethals from our screen were crossed to *Df(2R)nap^4* (R. KREBER and B. GANETZKY, unpublished data) that deletes the 41E5,F1-42A14,B1 region. Altogether, 23 independent lethals representing 10 complementation groups were uncovered by the *Df(2)nap^4* deletion. One of the complementation groups, a possible "hot spot," had nine independent alleles, one locus had three alleles, three other loci had two alleles each and five loci were represented by single alleles only. If the 23 *P*-induced mutants within the *Df(2)nap^4* deficiency identify 10 complementation groups, the 1748 mutants on the entire second chromosome would define 739 complementation groups, representing only about 37% of the estimated 2000 lethally mutable genes in this chromosome. Considering the existence of "hot spots" and the nonrandom distribution of *P* insertions, the 37% saturation is a more realistic value than the 58% calculated above on the basis of the Poisson distribution. It has been demonstrated that for a significant portion of the *X* chromosome the number of complementation groups is approximately half the number of polytene chromosomal bands (PERRIMON, ENGSTROM and MAHOWALD 1989). If this is true for the second chromosome as well, the level of saturation we attained is certainly higher than 37%. It is likely that our collection of lethals comprises all the "hot spots" and a significant part of the low frequency integration sites available

for the *P* element on the second chromosome.

Lethal phenotypes: Similarly to previously described observations (SHEARN *et al.* 1971; STEWART, MURPHY and FRISTROM 1972; KISS *et al.* 1976) various abnormalities were noticed in the 280 dissected late lethal lines. More than half of the strains displayed some disc abnormalities: the discs were frequently smaller than normal and/or showed aberrant folding. The typical "small disc" and "discless" phenotypes were also observed in our lethal collection.

We also identified 16 overgrowth mutations causing hyperplasia or neoplasia in one or more of the presumptive adult organs (Table 2). Seven of these display overgrowth of the hematopoietic organs (lymph glands) and can be classified as Class 2 *air* mutants according to WATSON, JOHNSON and DENELL (1991). Four lines exhibit imaginal disc overgrowth of the epithelial type (BRYANT and SCHMIDT 1990). One line displays overgrowth of the brain hemispheres. Four other lines show different combinations of the above phenotypes (Table 2).

In all the above mutants both penetrance and expressivity are rather varying, and the mutant phenotype develops gradually during a prolonged larval period. This variability can be explained by uncontrolled variations in the genetic background (SPARROW 1978) and/or the culture conditions as suggested earlier (SANG and BURNET 1963; COOKE and SANG 1972).

Allelism among the overgrowth mutants: Based on the complementation tests, a surprisingly low rate of allelism was found among the overgrowth mutants. As was previously observed, the insertion of *P* elements can induce a wide variety of hypomorphic and amorphic mutants (TSUBOTA, ASHBURNER and SCHEDL 1985). This raises the possibility that our overgrowth mutants may have independent alleles in the other lethal classes as well. Data from testing our strains over the above-mentioned $Df(2)nap^4$ deficiency are in agreement with this possibility. A locus represented by nine independent alleles was found, and the alleles belonged to the prepupal, pupal, pharate adult and semilethal categories.

A more detailed genetic and molecular analysis of the overgrowth mutants is in process. The rest of our lethal strains are being characterized in the frame of the Drosophila Genome Project. The mutations described in this work are available from the authors, while the rest of the lethal mutations are accessible through the Drosophila Genome Project.

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