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Isolation and Characterization of X-linked Lethal Mutants Affecting Differentiation of the Imaginal Discs in *Drosophila melanogaster*

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Summary. Twenty-seven late larval or early pupal lethal mutations were isolated for the X-chromosome, some of which showed structural and/or functional deficiencies of the imaginal discs. The mutants were grouped according to the size and morphology of their discs as follows: 1. discs normal: 18 mutants. 2. discs small: 2 mutants. 3. discs degenerate: 4 mutants. 4. discless: 1 mutant. 5. discs heterogeneous: 2 mutants. Preliminary characterization of the mutants included a study of disc morphology, puparium formation and pupal molt, *in vivo* and *in vitro* evagination of the imaginal discs, autonomy of the mutation in the disc tissue (differentiation after transplantation and gynander mosaicism test). Possible relations between disc morphology and the former characteristics are discussed.

Introduction

Differentiation of imaginal discs of *Drosophila melanogaster* has attracted the growing interest of developmental biologists since the pioneering work of Hadorn and his associates (Hadorn 1961). The system offers unique advantages for studying basic problems of development, *e.g.* determination, competence, hormonal control, cell interactions and morphogenesis (for a review, see Ursprung and Nöthiger 1972). Much has been done to elucidate the basic parameters of the system. The structure of the mature discs and morphological features of their development have been described at both the light and electron microscopical levels (Poodry and Schneiderman 1970; Ursprung 1972; Fristrom and Fristrom 1975). The final steps of disc development can be produced *in vitro* under the influence of ecdysones (Mandaron 1971; Fristrom *et al.* 1973). Efforts have been made to elucidate the biochemical events during the early phases of disc morphogenesis and differentiation (Fristrom 1972). Finally, the whole arsenal of mutants and genetical techniques available with *Drosophila* lends itself to a genetical approach unmatched in any other developmental system in animals (Postlethwait and Schneiderman 1973).

The recovery of mutants specifically affecting imaginal discs would facilitate the study of developmental processes in discs. Since imaginal discs are not needed for larval development but are required for adult development, mutants affecting imaginal discs have been systematically recovered by selecting for late larval or early pupal lethals (Shearn *et al.* 1971; Stewart *et al.* 1972). Using such an approach, Shearn and his co-workers (1971) recovered a large number of third chromosome late acting lethals. Using complementation tests and assuming that recovery of mutants in different genes follows a Poisson distribution, Shearn (1974) has estimated that the third chromosome has about 200-400 such genes. The number of X-linked mutants (26) recovered by Stewart *et al.* (1972) and the difficulty in performing complementation tests using such lethals have precluded a similar analysis for the X-chromosome.

We have isolated 27 new X-linked late acting lethals, some of which show obvious deficiences in the development of the imaginal discs. We report here a characterization of the mutants that includes studies on disc morphology, developmental capacity (puparium formation, evagination of the discs *in situ* and *in vitro*), autonomy (differentiation of mutant disc tissue in gynanders or after transplantation to wild-type larvae) and map position.

Materials and Methods

Maintenance of *Drosophila* stocks. A corn meal-agar medium was used in half litre bottles or glass vials. Stock cultures were kept at 18 °C, experimental cultures at 25 °C and 60 % relative humidity.

Mutagenesis and mutant selection. Lethal mutants on the X-chromosome were selected as follows. Two-day old y w cv sn^3 males (for a description of the mutants, see Lindsley and Grell 1968) were mutagenized

by feeding 0.025 M ethyl methane sulfonate (EMS) in a 1 % sucrose solution for 1 day (Lewis and Bacher 1968). Mutagenized males were mated to 5 or 6 Basc females each. The resulting y w cv sn^3/Basc female progeny carrying potentially mutated X-chromosomes were pair-mated to Basc males. The offspring were examined for the absence of y w cv sn^3 males indicating a recessive X-linked lethal mutation. To allow easier maintenance of mutant stocks the Basc balancer chromosome was subsequently replaced by Binsn (Binsn/Binsn females are sterile). The y w cv sn^3 male (hemizygous) larvae carrying a lethal (l) mutation can be recognized by the yellow phenotype.

Morphological characterization of the mutants. Experimental cultures were started with eggs collected during a 6 hr. period. On the 2nd and 3rd days (larval age is given throughout the paper as days after hatching) some light yeast suspension was added to ensure good feeding and synchronous larval development. Yellow larvae were selected on the 4th day, transferred to new food and kept at 25 °C. 4, 6 and 8 day old larvae were dissected in *Drosophila* Ringer's (Ephrussi and Beadle 1936) with watchmaker's forceps and tungsten needles. Gross morphology of the main anterior discs (eye-antenna, leg, wing, haltere) and the larval organs was examined through a dissecting microscope at 25×-100× magnification. Pupae were dissected 1 day following puparium formation to check for the normality of the pupal molt and for *in vivo* evagination of the discs.

In the case of our mutant designated l(1)discless-1, disc tissue was sought in 1 µ thick microscopical sections. Larval preparations were fixed in glutaraldehyde-osmium, embedded in Durcupan, sectioned with a Reichert FC2 microtome and stained with toluidine blue.

Determination of cell death. This was carried out according to the method of Spreij (1971): freshly dissected discs were stained for 5 minutes in 1.6×10^{-6} M acridine orange dissolved in Ringer's (pH 7-7.2) and examined through a Zeiss Jena fluorescent microscope (HBO 200 lamp, BG-12; 2 g prefilters and one OG 4 filter). Dead cells showed a bright fluorescence.

In vitro evagination. We used a modified version of a technique previously described (Fristrom *et al.* 1970). Freshly dissected leg and wing discs were thoroughly rinsed with sterile *Drosophila* Ringer's and transferred to 25 µl drops of Robb's medium (Robb 1969) containing 1 µg/ml ecdysterone as well as 100 µg/ml penicillin and streptomycin. Ecdysterone was purchased from Rohto Pharm. Co. or isolated from *Polypodium vulgare* according to the method of Jizba and Herout (1967). The culture drops were placed on siliconized depression slides and incubated at 25 °C in a humid chamber overnight. Discs of y w cv sn^3 male larvae were used as controls. Mutant discs were taken from 4, 6 and - when possible - 8 day old larvae. Discs from at least 10 larvae were used for each age group. One culture contained 3-4 discs from the same larva.

Genetic mapping. Lethal mutations were mapped using y w cv sn^3 and B chromosomes. Map positions proximal to sn^3 should be taken as only approximations because of the probable high frequency of double crossing over. A more precise determination of the map positions is in progress.

Autonomy tests. Study of the autonomy of the lethal mutations was restricted to the adult epidermis including the imaginal disc derivatives and the abdomen. In our mutants, the lethal mutation possibly causes the death of homozygous or hemizygous adult epidermal cells and so prevents their secreting an adult type of cuticle. When the lethality is expressed autonomously, death of the mutant epidermal cells occurs even in the presence of wild-type tissue. If the mutation is expressed in a non-autonomous way, mutant cells survive in the presence of wild-type tissue, indicating that they can be rescued by interacting with normal cells. In our case, animals carrying mutant and wild type tissue were produced as true genetic mosaics (gynanders) or by transplanting disc tissue of mutant phenotype into normal wild-type larvae.

A. Transplantation

Discs were dissected from mature lethal larvae in sterile Robb's medium, rinsed with the same medium, cut into pieces and each piece was injected into a mature wild type (Oregon R) larva through a glass capillary needle (Ephrussi and Beadle 1936). Implants were recovered from the adult hosts 4-6 days after eclosion, mounted between coverslips in Faure's solution and examined at 160× - 400× magnification.

B. Gynander mosaicism test

Gynander mosaics were induced using an unstable ring X- chromosome R(1)2 (Brown and Hannah 1952) obtained from Dr. A. Garcia-Bellido (C.S.I.C., Madrid, Spain). Heterozygous females (y w cv sn^3 l/Binsn) were crossed to R(1)2/Yy$^+$ males and the F_1 adult progeny were scored for gynanders carrying y w cv sn^3 l or Binsn hemizygous patches in the imaginal disc derivatives and the abdomen. Assuming that ring X instability is equally displayed in both kinds of heterozygous female progeny carrying y w cv sn^3 l or Binsn chromosomes, the ratio of gynanders with a y w cv sn^3 l hemizygous patch to those with a Binsn patch is constant and independent of the actual frequency of ring X-loss (Bryant and Zornetzer 1973). This ratio of gynanders was determined in control crosses of y w cv sn^3 females to R(1)2/Yy$^+$ males and used for the computation of the expected maximal number of gynanders carrying the y w cv sn^3 l chromosome.

Results

Recovery of mutants

Among the offspring of 40 males, we recovered 1957 females carrying a mutagenized y w cv sn^3 X-chromosome. 289 (14.8 %) of these females had no offspring, 798 (40.8 %) carried non-lethal X-chromosomes and 869 (44.4 %) had X-linked recessive lethals. Of this latter group, 27 mutants (1.4 %) had a lethal phase during the late larval or early pupal stage.

Grouping and morphological characterization of the lethal mutants

The mutants were tentatively grouped according to the size and morphological appearance of their imaginal

discs (Table 1). For this purpose we adapted, with some modifications, the nomenclature proposed by Stewart, Murphy and Fristrom (1972). According to this, description of a recessive lethal is "initiated by the letter 'l', followed in parentheses by the chromosome, then by one of the descriptive abbreviations, and finally by a number identifying a particular locus" (Stewart *et al.* 1972). The following categories were used to classify the mutants:

discs normal
discs small
discs degenerate
discless
discs heterogeneous.

Although morphological characterization referred mainly to the imaginal discs, other larval organs were included as well.

discs normal (d. norm.) mutants. When dissected from mature larvae (for the exact time, see Table 1), the imaginal discs appear to have a size and structure similar to that of wild-type discs from mature 3rd instar larvae. There are only two exceptions where discs have normal size but abnormal structure, as follows:

l(1) d. norm.-12 has completely normal-looking discs on the fourth day. This mutant is not able to form a puparium, although the larvae survive up to 10-12 days. Starting on the 6th day, all the discs become abnormal: the peripodial membrane becomes enormously distended and a highly distorted, partially evaginated structure becomes visible in the disc lumen (Fig. 1,C).

l(1) d. norm.-27 has discs of normal size, but their structure is abnormal, sometimes without any recognizable folding pattern. The degree of disc abnormality varies widely even within the same animal.

discs small (d. sml) mutants: discs of mature mutant larvae are substantially smaller than those of the wild type. Their folded structure seems to be very undeveloped, resembling discs of early third instar larvae. l(1) d. sml-9 does not form a puparium. The discs grow slowly throughout the whole life of the surviving larva and, by the 9th or 10th day, they become comparable in size to the mature wild-type discs.

discs degenerate (d. deg.) mutants: only very small fragments or no disc tissue at all can be found upon dissection. The fragments have no visible internal structure.

discless mutant: no disc tissue was detected either by dissection or microscopic examination of serial sections of mature (8 day old) larvae. Microscopic examination of sections of 40 h old larvae gave the same result.

discs heterogeneous (d. het.) mutants: the discs are of normal size and structure, except leg discs which are small (d. het.-1) or degenerated (d. het.-2, Fig. 1,E).

The amount of fat body tissue (a central organ for biochemical metabolism and storage of reserve materials) is reduced in all of the mutants (including discs normal ones). l(1) d. norm.-13 larvae show an extreme reduction, having only a few fat body fragments (transparent "glass larvae").

Rate of development of the mutants

In spite of the optimal rearing conditions, hemizygous male mutant larvae develop more slowly than their heterozygous siblings: out of 27, only 2 mutants (7.4 %) form puparia on day 4 and 1 (3.7 %) on day 5. 16 mutants (59.2 %) pupariate on day 6, 2 (7.4 %) between days 8-10 and 6 mutants (22.2 %) make no puparia at all (Table 1). At the end of the 4th day most of the mutant larvae are smaller and much less developed than their normal heterozygous counterparts which pupariate at that time. However, they reach the normal size of a mature 3rd instar larva by the time of their puparium formation. There is no obvious correlation between disc morphology and the rate of development.

Lethal period

Six mutants (22.2 %) do not pupariate and die as larvae. Larval life can be prolonged substantially in these cases (Table 1). Twenty-one mutants (77.8 %) form puparia; four of them (14.8 %) die as prepupae while the rest (70.4 %) die within 1 day following the pupal molt. The inability to form puparia is relatively frequent in groups having deficient discs (Table 1).

Map position of the mutant loci

For the preliminary characterization, we mapped our mutants relative to y, w, cv, sn^3 and B. As there were no markers between sn^3 and B, mapping of the lethals in this region was only approximate because

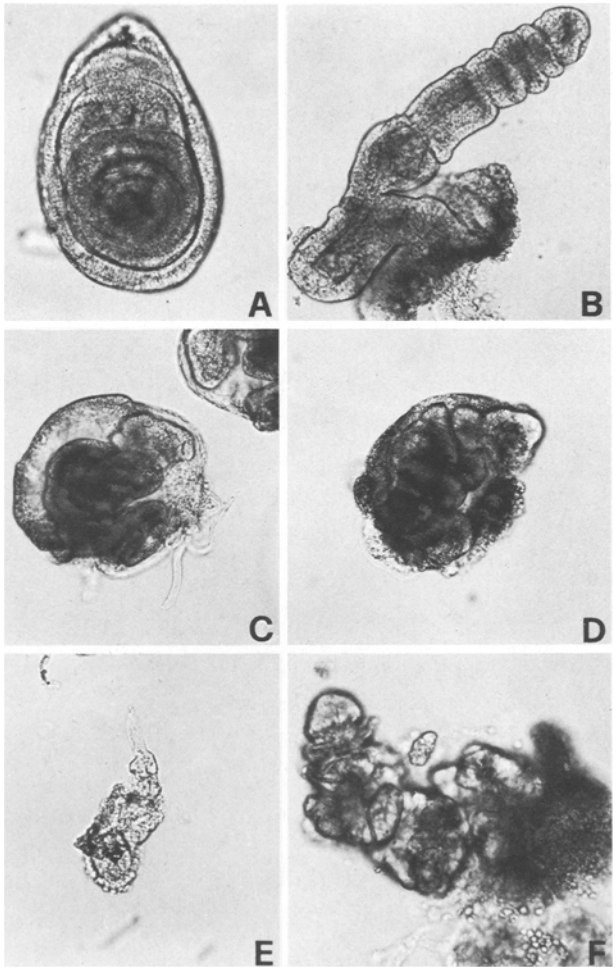

Fig.1. Morphological appearance of leg imaginal discs from mature 3rd instar larvae: A - wild type, freshly dissected, 500 ×; B - wild type, after *in vitro* evagination, 400 ×; C - l(1)d.norm.-12, freshly dissected, 400 ×; D - l(1)d.norm.-12, after *in vitro* evagination, 400 ×; E - l(1)d.het.-2, freshly dissected, 500 ×; F - l(1)d.norm.-24, after *in vitro* evagination, 400 ×

of double recombination. Map positions of the mutants are given in Table 1 and compared with those of Stewart *et al.* (1972) in Fig.2.

Evagination of the imaginal discs

Both *in vivo* and *in vitro* evagination of the mutant discs were tested. (See Materials and Methods.) For the *in vitro* evagination test, leg discs were generally used.

In vivo and *in vitro* evagination correlate well with disc size (Table 1). Discs of most of the discs normal mutants evaginate normally *in vivo* or *in vitro*. However, there are several exceptions. l(1) d. norm.-12 forms highly distorted leg structures resembling partial evagination in the tarsal region (see Fig.1,C). Such a condition was found *in vivo* in discs of 5 day and older larvae (see the Morphology section). This condition was also found *in vitro* by culturing normal looking discs from 4 day old larvae with ecdysterone (1 μg/ml) (Fig.1,D). l(1) d. norm.-27 shows a distorted, partial evagination *in situ*, frequently with some of the disc derivatives missing. There is no *in vitro* evagination at all. l(1)d. norm.-21, -24, -25, -26 and -28 show a normal *in vivo* evagination of their discs. However, the first 3 produces morphological irregularities *in vitro*, and the last two gave no *in vitro* evagination at all (Fig.1,F).

Discs small and discs degenerate mutants do not show evagination either *in vivo*, or *in vitro*. In discs heterogeneous mutants, leg discs are small or degenerated and all the other discs are normal. Accordingly, leg discs do not evaginate either *in vivo* or *in vitro* while wing discs evaginate normally in both cases (Table 1).

Cell death in the imaginal discs

The occurrence of dead cells is more frequent than in wild type discs, even in discs normal mutants (Table 1). All of the discs small and discs degenerate mutants show a high or extremely high level of cell death. In discs heterogeneous mutants, cell death is highly elevated in the leg discs and normal or near to normal in wing discs.

Transplantation of the imaginal discs

In all the cases examined, transplanted mutant disc tissue secreted an adult cuticle during the metamorphosis of the host (Table 1). This means that none of the lethal mutations tested are expressed autonomously in the disc tissue. In the case of discs normal mutants the implant usually secreted a normal adult cuticle equipped with numerous bristles and hairs. In two mutants (l(1) d. norm.-11, -28), however, the secreted cuticle had very few bristles and hairs. l(1) d. sml.-9, the only mutant tested outside the discs normal group, gave a similar result (Table 1).

Gynander mosaicism

a) Control: $y\,w\,cv\,sn^3/Binsn$ ♀♀ were mated to $R(1)2/Yy^+$ ♂♂. There was no lethal allele on the

Table 1. Characteristics of the late lethal mutants

Mutant	Age in days of larvae at puparium formation	Lethal phase	Cell death	Evagination *in vivo*	Evagination *in vitro*	Differentiation in transplants	Map position
l(1)d.norm.							
-11	9[a]	larval	increased	0	0	±	26
-12	4[a]	larval	increased	dt	dt	+	0.2
-13	10	prepupal	normal	+	+	N.D.	18.5
-14	8	pupal	normal	+	+	+	N.D.
-15	6	pupal	increased	+	+	+	9.5
-16	6	pupal	normal	+	+	+	N.D.
-17	4	pupal	normal	+	+	+	N.D.
-18	6	pupal	normal	+	+	+	N.D.
-19	6	pupal	normal	+	+	+	0.8
-20	6	pupal	increased	+	+	+	N.D.
-21	5	pupal	normal	+	dt	+	35.8
-22	6	pupal	normal	+	+	N.D.	N.D.
-23	6	pupal	normal	+	+	+	N.D.
-24	6	pupal	normal	+	dt	+	0.3
-25	6	pupal	increased	+	dt	+	22
-26	6	pupal	normal	+	0	+	56-58
-27	6	pupal	increased	dt	0	+	41.9
-28	6	pupal	normal	+	0	±	61.9
l(1)d.sml.							
- 8	6[a]	larval	increased	0	0	N.D.	19.5
- 9	6[a]	larval	increased	0	0	±	56.9-57.1
l(1)d.deg.							
- 9	6	prepupal	greatly increased	0	0	N.A.	29.7
-10	6	prepupal	"	0	0	N.A.	46.7
-11	6	prepupal	"	0	0	N.A.	27.2
-12	4[a]	larval	increased	0	0	N.A.	65.8
l(1)discless							
- 1	8[a]	larval	N.A.	N.A.	N.A.	N.A.	35
l(1)d.het							
- 1	4	pupal	increased(0)[b]	0(+)[b]	0(+)[b]	+(+)[b]	27.5
- 2	6	pupal	greatly increased(0)[b]	0(+)[b]	0(+)[b]	N.D.(+)[b]	56.8-57.2

[a] For non-pupariating lethals, the time required to reach the size of the normal mature 3rd instar larva is given.
[b] Data in brackets refer to wing discs.
Abbreviations used: 0, absent; dt, distorted; +, like wild type; ±, much less than wild type; N.A., not applicable; N.D., not determined.

y w cv sn^3 chromosome so all the progeny classes, including gynanders, were viable as adults. The distribution of progeny is shown in Table 2.

It is particularly noticeable that there is an inequality in the numbers of progeny carrying Binsn or y w cv sn^3 chromosome. This difference is consistent from experiment to experiment and may result from the lower viability of animals carrying the Binsn chromosome. As the same inequality is expressed in the ratio of gynanders carrying Binsn or y w cv sn^3 hemizygous patches, we assume that the suspected difference in viability is expressed in gynanders as well. Consequently, Binsn-carrying gynanders could be used as an internal control in those crosses where the y w cv sn^3 chromosome carries a lethal allele, provided that the lethality has no influence on the frequency of Binsn-carrying gynanders (Bryant and Zornetzer 1973). The ratio of y w cv sn^3 gynanders to Binsn gynanders was found to be 1.7. This factor was used to compute the "estimated total number" of y w cv sn^{3}l gynanders throughout the experiments.

b) Experimental: Results of y w cv sn^{3}l/Binsn ♀♀ × R(1)2/Yy$^+$ ♂♂ crosses are summarized in Table 3. In the majority of cases, no adult gynanders with y w cv sn^{3}l

Table 2. Distribution of progeny from y w cv sn^3/Binsn♀ × R(1)2/Yy$^+$♂

Female			Male			
Binsn/R(1)2	Gynanders with Binsn patches	y w cv sn^3/R(1)2	Gynanders with y w cv sn^3 patches	Binsn/Yy$^+$	y w cv sn^3/Yy$^+$	XO♂ (y w cv sn^3/0)
172	161	102	279	1960	2029	845

Table 3. Results of the gynandromorph mosaicism test

Mutant	Binsn/R(1)2 gynanders found	Lethal-bearing gynanders Estimated total number	Found	Gynander viability
l(1)d.norm.-11	64	109	none	< 0.009
l(1)d.norm.-12	38	65	none	< 0.015
l(1)d.norm.-13	41	70	none	< 0.014
l(1)d.norm.-14	22	37	3	0.080
l(1)d.norm.-28	18	31	3	0.097
l(1)d.sml.-8	54	92	none	< 0.010
l(1)d.deg.-9	66	112	none	< 0.009
l(1)d.deg.-12	61	104	none	< 0.009
l(1)discless-1	28	48	none	< 0.021
l(1)d.het.-1	230	391	17	0.043
l(1)d.het.-2	24	41	3	0.073

patches were found, even if their expected number was more than 100. These mutations either are autonomous for the adult epidermal tissue or the viability of the gynanders is extremely low. Four strains out of eleven tested gave adult gynanders. Although the number of gynanders found was much less than expected, these four strains are clearly nonautonomous. It is likely that the frequency of recovery of gynander patches is different for different parts of the adult. This possibility is especially interesting with respect to the "discs heterogeneous" mutants. More detailed studies on the distribution and size of y w cv sn^{3}l patches in gynanders are in progress.

Discussion

The selection scheme we used for isolating mutants with imaginal disc deficiencies is similar to that of Shearn *et al.* (1971) and Stewart *et al.* (1972) and is based on the assumption that normal development of the imaginal discs is not necessary for normal development of the larva. In general, such late larval and early pupal lethals are a rich source of mutants with various disc abnormalities. In the present study we isolated 27 late lethals out of 1957 mutagenized X-chromosomes (1.4 %). This recovery is higher than that in a recent study (Stewart *et al.* 1972) where 26 (0.61 %) lethals were recovered out of 4294 mutagenized X-chromosomes. The difference could be explained by the fact that, in our experiments, no special emphasis was placed on larval viability and developmental time of the mutants. The larval lifetime is generally longer in our mutants than in wild type. About half of the mutants (13 out of 27) do not show any obvious disc deficiency.

We have tentatively grouped our mutants according to the size and morphology of their imaginal discs, as proposed earlier (Stewart *et al.* 1972). Since mutations in *Drosophila* are frequently expressed in a pleiotropic way (Stern and Tokunaga 1968), any of the mutant characteristics could be arbitrarily chosen as a basis for classification (*e.g.*, l(1) discless-1 has no discs, does not form a puparium and has an abnormally small amount of fat body). We feel that developmental mutants should ultimately be categorized according to the primary focus/foci of expression of the mutant gene function, *i.e.* according to the sites where the mutation is expressed autonomously. A classifi-

cation of this type would obviously need a penetrating study particularly with respect to autonomy.

In the following sections we will survey the results and discuss some of the interesting points. discs normal mutants: the discs of 8 of our 18 mutants appear to be normal. However, larval development is prolonged (on the average, puparium formation occurs 6.2 days after hatching while that in the y w cv sn^{3}1/ Binsn heterozygous control occurs on day 4). In these cases we conclude that the mutant gene is not disc-specific at all and prolongation of larval development as well as lethality is caused by malfunctioning of other larval organs.

The rest of the mutants in this group show enhanced cell death or deficiencies of evagination, the latter mostly *in vitro*. There are three mutants having serious defects: l(1) d. norm.-11, l(1) d. norm.-12 and l(1) d. norm.-27 (Table 1). According to the transplantation and gynander-mosaicism data, l(1) d. norm.-11 could be autonomous for the imaginal disc tissue in respect of cell death or inability to synthesize an adult type of cuticle. Slow larval growth and inability to form puparia can be regarded as pleiotropic effects. l(1) d. norm.-12 develops normally up to the 4th day. However, the larvae do not pupariate, and serious morphological distortions of the discs as well as enhanced cell death develop by the 6th day. Since transplanted disc fragments differentiate normally, cell death or inability to reach terminal differentiation (secretion of a cuticle of the adult type) can not be an autonomous feature. In l(1) d. norm.-27 abnormal evagination *in vivo* of the discs is especially prominent, but disc fragments differentiate in transplants. Perhaps distortions of disc structure and inability to evaginate would behave autonomously in gynander larvae for both of these mutants. Investigations on this possibility are in progress in our laboratory.

In three other mutants showing a more or less distorted in vitro evagination (l(1) d. norm.-21, -24 and -25), all the other characteristics are normal including differentiation after transplantation. Deficiency of *in vitro* evagination probably reflects an inadequacy of the *in vitro* environment for the mutant discs, which may be more sensitive than the wild type.

Imaginal discs of l(1) d. norm.-28 show a completely normal cell death pattern and evaginate *in vivo* but not *in vitro*, and differentiation in the transplantation test is poor. Gynanders from this strain (Table 3) carrying y w cv sn^{3}1 patches in the anterior disc derivatives had shortened, crippled legs and wings with fewer bristles. This mutation may autonomously interfere with the final differentiation of disc derivatives. The mutation is probably not lethal to individual disc cells or dead cells can be replaced by regeneration. discs small and discs degenerate mutants: the difference between the two groups is mostly qualitative rather than quantitative. Within these groups the mutants show more uniform characteristics than those in the discs normal group. Frequency of cell death is much higher than normal (Table 1). Discs do not evaginate either *in vivo* or *in vitro*. Death occurs in old larvae or prepupae. Inability to perform the pupal molt is probably connected with the lack of evagination. Discs of l(1) d. sml.-9 gave a partial differentiation in the transplantation test. No gynanders have been found in 3 mutants tested so far (Table 3). Cell death in disc tissue caused by the mutation is probably expressed in an autonomous way and can be responsible for the degeneration of the imaginal discs. However, more detailed investigations are needed with respect to autonomy. The development of these mutants needs to be studied to determine whether development of the disc tissue starts normally and degeneration occurs later or whether development has been hindered from the beginning by cell death. Some of these observations coincide with those made by other authors on similar mutants (Stewart *et al.* 1972; Murphy 1974).

discless mutant: one mutant was recovered having apparently no disc tissue at all. Since it is easy to distinguish the small, cuboidal, undifferentiated imaginal disc cells from other larval cells (Ursprung 1972), a critical test for the presence or absence of disc tissue is the microscopical examination of sections made of appropriate regions of the larva. By this method, no disc cells could be detected in mature 3rd instar as well as young 2nd instar larvae. Similar discless mutants have been reported earlier among lethals for the 3rd chromosome (Shearn *et al.* 1971). However, no microscopical examinations were made in this case. On the other hand, Stewart *et al.* (1972) and Murphy (1974) were able to find, by microscopy, some highly degenerated remnants of disc

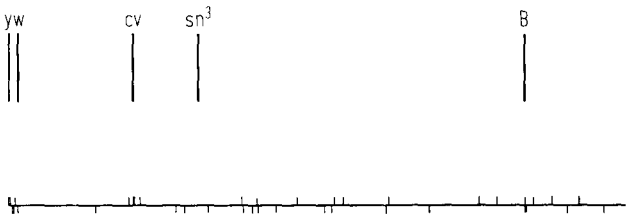

Fig.2. Approximate map position of late lethal mutants on the X-chromosome. Data from Stewart *et al.* (1972) - upper row - and from our laboratory - lower row - are represented

tissue in each of their mutants which appeared to be discless upon dissection.

Theoretically, the discless phenotype can arise by two mechanisms: either no disc tissue is formed at all, or disc cells differentiate at the proper time and degenerate later. In the first case, the mutant gene should function during early embryogenesis, when presumptive imaginal disc cells are "singled out" (Nöthiger 1972). If those hypothetical maternal factors which are supposed to play a decisive role in the primary determination of nuclei during blastoderm formation were involved, the mutant phenotype should be inherited maternally (Rice 1973, quoted by Murphy 1974; Rice and Garen 1975). This is clearly not the case here. Another possibility is a failure of invagination of the presumptive disc tissue from the blastoderm, which would also result in a complete lack of imaginal discs, as supposed by Murphy (1974). In the case of a secondary degeneration of the preexisting disc tissue, the mutant phenotype could be expressed autonomously or non-autonomously. To distinguish between the two possibilities, critical tests using embryonal transplantation (Shearn and Garen 1974), as well as X-ray induced somatic recombination (Russell 1974), are in progress with l(1) discless-1. discs heterogeneous mutants: unlike the mutants discussed earlier, in which all the discs are uniformly influenced by the mutation, only leg discs of these strains show the characteristics of discs small or discs degenerate mutants, while the other discs appear to be normal (Table 1). The difference among the discs is expressed invariably and consistently. Shearn *et al.* (1971) isolated similar mutants on the 3rd chromosome and Murphy (1974) mentioned a mutant on the X-chromosome in which discs are differentially influenced by the mutation. In these cases the mutant gene seems to be specific for a certain kind of disc, *i.e.*, for the leg discs in our mutants.

As shown in Table 1, none of the 19 mutants tested proved to be autonomous in the disc transplantation test, *i.e.* all of them differentiated at least some adult cuticle. In other words, none of the tested mutants is completely a cell-autonomous lethal for the adult epidermis or incapable of secreting an adult cuticle. With some of the mutants, we made another test for autonomy using gynander mosaics. As Table 3 shows, four of the eleven mutants tested gave gynanders and proved to be non-autonomous. However, the remaining seven strains have not yielded adult gynanders so far. We think this is because of the low viability of the gynanders: the hemizygous male part of the body is probably large enough to include the lethal focus, or a part of it which is enough to prevent development of the gynander fly. In this case, we think, transplantation results are more reliable and prove the non-autonomy of the mutants tested.

Based on complementation data from EMS-induced late-acting lethals, Shearn (1974) has recently made an estimation of the total number of genes causing late lethality. Assuming a random distribution throughout the genome, there are 364 such genes on the third chromosome, about 900 in the entire genome, and about 180 such genes on the X-chromosome. Up to now, 53 late acting lethals on the X-chromosome have been isolated using EMS-mutagenesis, 26 by Stewart *et al.* (1972) and 27 in this laboratory.

An analysis similar to the above one would allow a direct estimation of the number of such genes on the X-chromosome. Unfortunately, complementation tests of lethal alleles on the X-chromosome are very difficult. Since no such data are available for the time being, we can rely only on the map positions and the phenotypic characteristics of the mutants to estimate the total number of X-linked late acting lethal genes. Fig.2 shows the distribution on the X-chromosome of the 39 mutants mapped. As can be seen, there are seven regions where the mutant alleles are close enough to be allelic. Allelic mutants should have similar phenotypes. After comparing the phenotypic characteristics of the mutants (see Tables 1 and 2 in Stewart *et al.* 1972 as well as Tables 1 and 3 in this paper), a supposition of allelism seems possible in five cases out of seven, *i.e.* 10 of the 39 mutants would

belong to 5 complementation groups while the remaining 29 would represent 29 other complementation groups. Assuming that our mutations represent a random sample of the genes on the X-chromosome and follow a Poisson distribution, we calculate the total number of genes to be 118. This estimate is in good agreement with the estimation made by Shearn (1974).

The growing number of mutants isolated in different laboratories raises the hope that, through these studies, we will gain a deeper understanding of the genetic mechanisms controlling imaginal disc development in *Drosophila*. This knowledge would be of immense importance in solving some of the basic problems of developmental biology as well as specific questions of insect development.

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