Proc. Nat. Acad. Sci. USA
Vol. 68, No. 10, pp. 2594–2598, October 1971

Imaginal Disc Abnormalities in Lethal Mutants of *Drosophila*

(genetic control/development)

ALLEN SHEARN*, THOMAS RICE, ALAN GAREN‡, AND WALTER GEHRING§

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

Contributed by Alan Garen, July 9, 1971

ABSTRACT Late lethal mutants of *Drosophila melanogaster*, dying after the larval stage of development, were isolated. The homozygous mutant larvae were examined for abnormal imaginal disc morphology, and the discs were injected into normal larval hosts to test their capacities to differentiate into adult structures. In about half of the mutants analyzed, disc abnormalities were found. Included among the abnormalities were missing discs, small discs incapable of differentiating, morphologically normal discs with limited capacities for differentiation, and discs with homeotic transformations. In some mutants all discs were affected, and in others only certain discs. The most extreme abnormal phenotype is a class of "discless" mutants. The viability of these mutant larvae indicates that the discs are essential only for the development of an adult and not of a larva. The late lethals are therefore a major source of mutants for studying the genetic control of disc formation.

The imaginal discs of a *Drosophila melanogaster* larva contain the cells which will form the epidermal structures of the adult fly during the subsequent developmental stages of pupation and metamorphosis (1). There are several pairs of discs, each characterized by its location in the larva, by its size and shape, and most significantly by the part of the adult which it forms (Fig. 1). At the onset of pupation, the number of imaginal cells in an individual disc ranges from a few hundred in the smaller discs to several thousand in the larger ones. All of the imaginal cells in a larva are cytologically similar, resembling the relatively undifferentiated cells of a young embryo. Not until pupation do the imaginal cells undergo the pronounced cytological changes that culminate in the appearance of highly specialized adult cells.

The differentiation of a disc into an adult organ is not dependent on its location in the larva. For example, an eye disc can be removed from its normal location in a donor larva and injected into the abdominal cavity of a host larva, where it forms fully differentiated eye structures when the host undergoes metamorphosis (2). Still more remarkable is the demonstration that a disc also can differentiate *in vitro* (3). This capacity for autonomous differentiation indicates that the discs of a mature larva are rigorously determined for their subsequent developmental fates.

This report is concerned with the genetic control of disc development. To identify relevant genes, we concentrated our attention on lethal mutants that die late in development, after the larval stage and before emergence of the adult. It was shown previously that the late lethal mutant *lethal giant larvae* produces fully-grown larvae having severely defective discs (4), suggesting that the discs might not be essential structures during the larval stage. We isolated 134 late lethal mutants and examined the homozygous mutant larvae for evidence of abnormal disc development. In nearly half of the mutants a variety of disc abnormalities were detected, including many not previously observed in *Drosophila*.

MATERIALS AND METHODS

Isolation of third-chromosome lethal mutants

Mutations were chemically induced in young adult males, by feeding solutions of ethylmethanesulfonate (EMS) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG), or by abdominal injection of the acridine compound ICR-170 (5, 6). Depending on the desired level of mutagenesis, the concentration was varied from 2.5 to 25.0 mM for EMS, from 6.8 to 13.6 mM for NG, and from 0.1 to 0.2 mg/ml for ICR. The males were homozygous for the recessive third-chromosome markers *ebony* (*e*) and *multiple wing hairs* (*mwh*), and, in some of the later experiments, also *red Malpighian tubules* (*red*) (7). The mutagenized males were mated to females with the dominant marker *Glued* (*Gl*) on one of the third-chromosomes, the other third-chromosome being the balancer *TM1*, which has several inversions to suppress crossingover and also the dominant eye

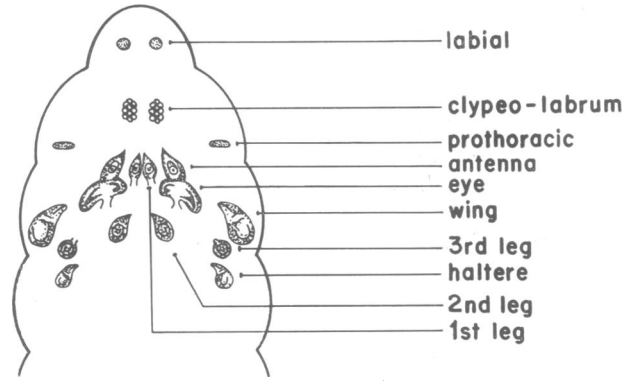

FIG. 1. The imaginal discs in the anterior region of a third-instar larva of *Drosphila melanogaster*. All of the discs analyzed in this report are found in this region. The principal discs not shown are the genital disc, which is in the posterior region, and the abdominal "histoblasts", which are in the middle and posterior regions. Each of the discs in the figure occurs as a pair, oriented with bilateral symmetry about the longitudinal axis of the larva.

* Present address: Biology Dept., Johns Hopkins University, Baltimore, Md. 21218.
‡ Reprint requests may be addressed to Dr. Alan Garen, Box 1937 Yale Station, New Haven, Conn. 06520.
§ Present address: Anatomy Dept., Yale University Medical School, New Haven, Conn. 06510.

marker *Moiré*. Progeny with the *Moiré* phenotype were isolated, and individual males were mated separately to females from the same balancer stock as used in the first cross. From each of the individual crosses, progeny with the *Moiré* phenotype (and thus containing the balancer and one mutagenized chromosome) were isolated and sibling males and females were mated to each other to produce progeny homozygous for the mutagenized chromosome. This scheme is summarized as follows:

1. mwh e/mwh e males × *Gl/TM1 females*
2. mwh e/TM1 males × *Gl/TM1 females*
3. mwh e/TM1 males × *mwh e/TM1 females*

The lethal mutants were identified by the absence of the phenotypes *ebony* and *multiple wing hairs* among the adult progeny of the third cross. Since *ebony* (and also *red*) is expressed homozygously as a larval and an adult phenotype, it also is possible, by examining the larvae in this cross, to identify which of the lethal mutants are late lethals; the presence of fully grown *ebony* larvae with darkended spiracles, or *red* larvae with red Malpighian tubules, indicates that death occurs later than the larval stage.

Tests for disc abnormalities

Homozygous mutant larvae were isolated from balanced heterozygous stocks of each late lethal mutant. The larvae were kept at 25°C for 4 days after hatching, at which time most had developed to an advanced third-instar stage. The larvae were dissected and examined microscopically (×37.5) for missing discs or discs of abnormal size or shape. Only the larger discs were routinely checked, including the wing, eye-antenna, haltere, and the three leg discs (see Fig. 1). These discs, if present, were tested for their developmental capacities by injecting each disc into the abdominal cavity of a young third-instar larval host (Oregon *R* wild-type strain). After 10 days at 25°C, the adult that developed from each larval host was dissected and any implant found in its abdominal cavity was prepared for microscopic examination (8). In all cases the tissue recovered from the abdomen of the host had the genetic markers *ebony* and *multiple wing hairs* derived from the injected mutant disc.

TABLE 1 *Results of isolating lethal mutants*

Mutagen	Chromosomes tested	Lethal mutants	Late lethal mutants
EMS	635	324	62
NG	1609	125	34
ICR	923	129	38

RESULTS

Among 3167 balanced stocks carrying a mutagenized third-chromosome, 578 were found to have recessive lethal mutations, and 134 of these behaved as late lethals (Table 1). All of the 134 late lethals were tested for disc abnormalities. None were detected in 71 of the mutants, which were not analyzed further. Some of these mutants could have non-autonomous disc defects, or defects in discs that were not tested. Among the other mutants, various kinds of disc abnormalities were found, in some cases affecting all the discs in a mutant and in other cases only certain discs. There are eight distinguishable classes of mutants (Table 2), as follows.

Class A—all discs are missing

The 10 mutants in this class produced homozygous mutant larvae that grew almost to the size of mature normal third-instar larvae but did not form any visible discs (Fig. 2). In addition to the larger discs routinely examined, the genital, prothoracic, and labial discs also were missing. Larval structures other than the discs appeared to be normal, except for the salivary glands and the brain lobes, which were small in some of the mutants. Puparium formation occurred with several of the mutants, but there was no evidence of differentiated imaginal tissue within the pupal cases.

Class B—most discs are missing and the others are small and developmentally blocked

The discs present in the six mutants in this class were substantially smaller than in normal larvae of the same age, and they were almost totally incapable of forming any of the characteristic adult structures. Because of these severe morphological and developmental abnormalities, it was difficult

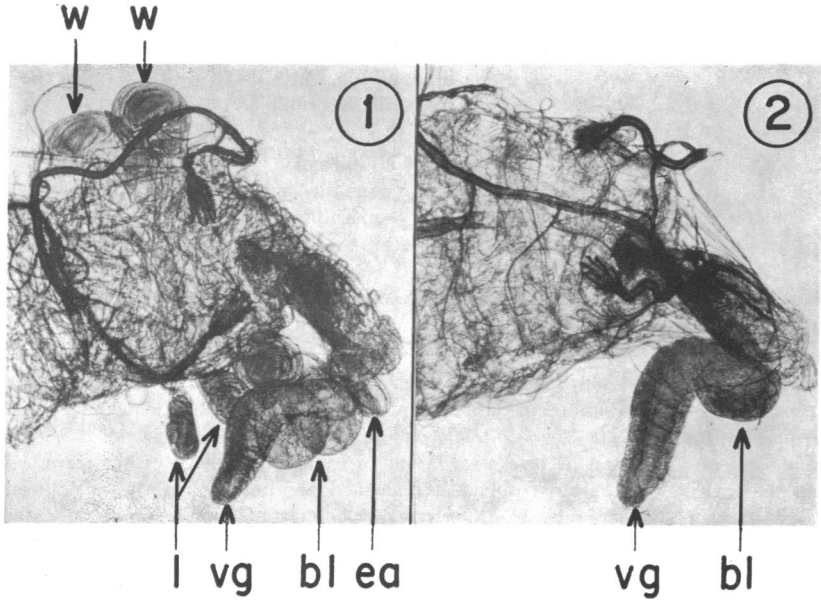

FIG. 2. Dissected anterior regions of a normal and a discless mutant (class *A*) larva. Several of the larval organs were removed and the specimens were arranged to obtain the best exposure of the discs. In the normal larva (panel *1*) the eye-antenna (*ea*), wing (*w*), and leg (*l*) discs are visible; in the mutant larva (panel *2*) there is no evidence of any discs being present. The two brain lobes (*bl*) are visible in the normal larva, but in the mutant one of the lobes is hidden under the other. The ventral ganglion (*vg*) is also visible. The normal and mutant larvae were of about the same overall size, corresponding to the third-instar stage reached at about 100 hr at 25°C. The magnification was ×45.

to establish the identities of the discs. An example of this type of defective disc, illustrating the characteristically reduced size and blocked developmental capacity, is shown in Fig. 3.

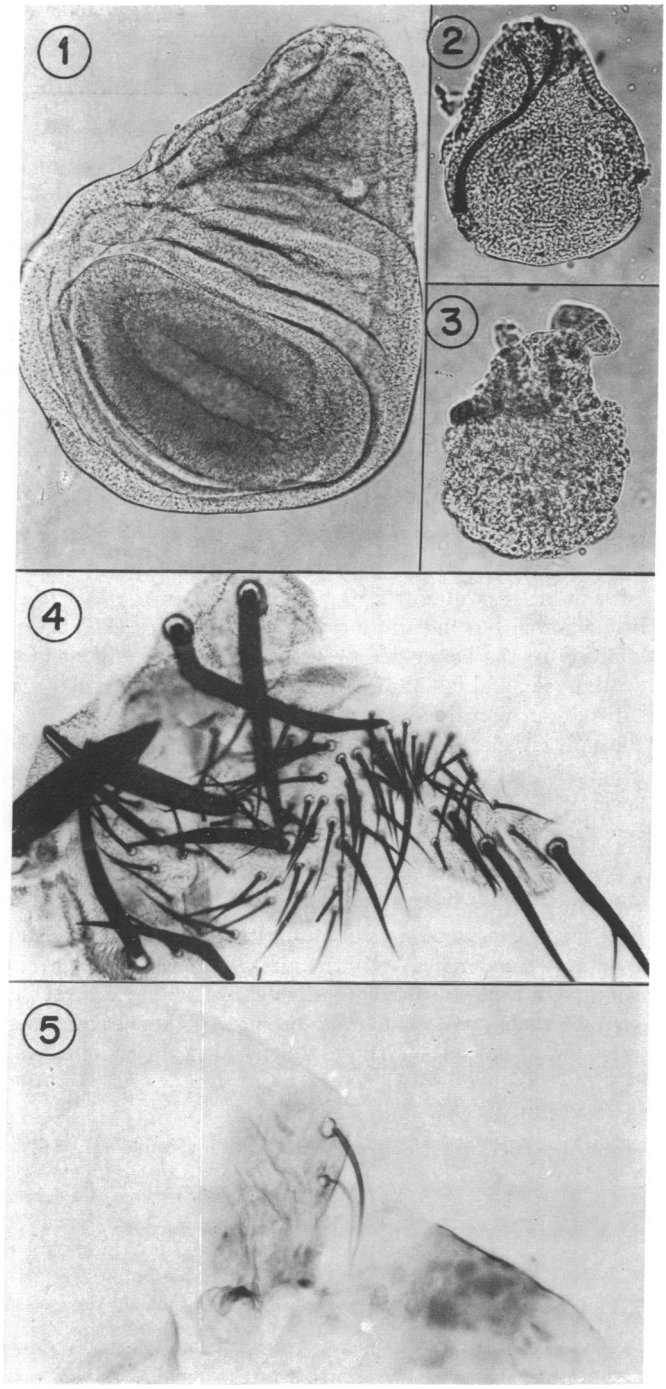

FIG. 3. An example of a developmentally blocked wing disc. *Panel 1.* Wing disc from a late third-instar normal larva. *Panel 2.* Wing disc from an early third-instar normal larva. *Panel 3.* Wing disc from a late third-instar mutant larva (class *C*). Panel *4.* Results of a developmental test (see *Materials and Methods*) of a wing disc from a late third-instar normal larva, showing some of the bristle structures formed; similar results were obtained with the smaller wing disc from an early third-instar normal larva. *Panel 5.* All of the structures formed in a developmental test of a mutant wing disc; only two bristles, which are incompletely pigmented, could be identified. The magnification was ×200.

Class C—all discs are present but all are small and developmentally blocked

The discs in the 10 mutants in this class were severely defective morphologically and developmentally (Fig. 3), similar to the discs present in the class *B* mutants.

Class D—all discs are present and morphologically normal but all are developmentally abnormal

In contrast to the developmentally blocked discs in the preceding mutants, the discs in the three mutants in this class were capable of producing recognizable patterns of adult tissue when tested in normal larval hosts. However, both abnormal and normal adult structures were formed, and some of the expected structures usually were absent (Fig. 4).

Class E—most discs are missing and the others are normal

The only discs consistently present in the two mutants in this class were the wing discs in one mutant and the three pairs of leg discs in the second mutant, all of which were morphologically and developmentally normal. The other discs usually were missing, but occasionally there were small discs visible that were developmentally blocked.

Class F—all discs are present but some are small and developmentally blocked

Several pairs of discs were defective in two of the mutants in this class, namely the wing, haltere, and eye discs in one mutant, and the three pairs of leg discs in the second. Only the antenna discs were defective in the third mutant. All of the other discs were normal. The affected discs had severe morphological and developmental abnormalities, similar to the discs in the mutant classes *B* and *C* (Fig. 3).

TABLE 2. *Late lethal mutants with imaginal disc abnormalities*

Mutant class		Discs formed	Developmental capacities	Number of mutants EMS	NG	ICR	Total
All discs affected	A	none	—	5	1	4	10
	B	some	all blocked	1	3	2	6
	C	all	all blocked	5	4	1	10
	D	all	all abnormal	1	1	1	3
Some discs affected	E	some	all normal	2	0	0	2
	F	all	some blocked,	2	1	0	3
	G	all	some abnormal	12	9	5	26
	H	all	some homeotic transformations,	0	1	2	3

The phenotypic characteristics of each mutant class are described in the text and in Figs. 2, 3, and 4. The developmental tests (see *Materials and Methods*) were done on the disc pairs from at least two larvae of each mutant. All of the discs that were developmentally blocked or abnormal in the initial tests were retested at least once. The morphological examinations were initially done on two or more larvae of each mutant, and for the mutants that showed morphological disc defects an additional eight larvae were subsequently examined.

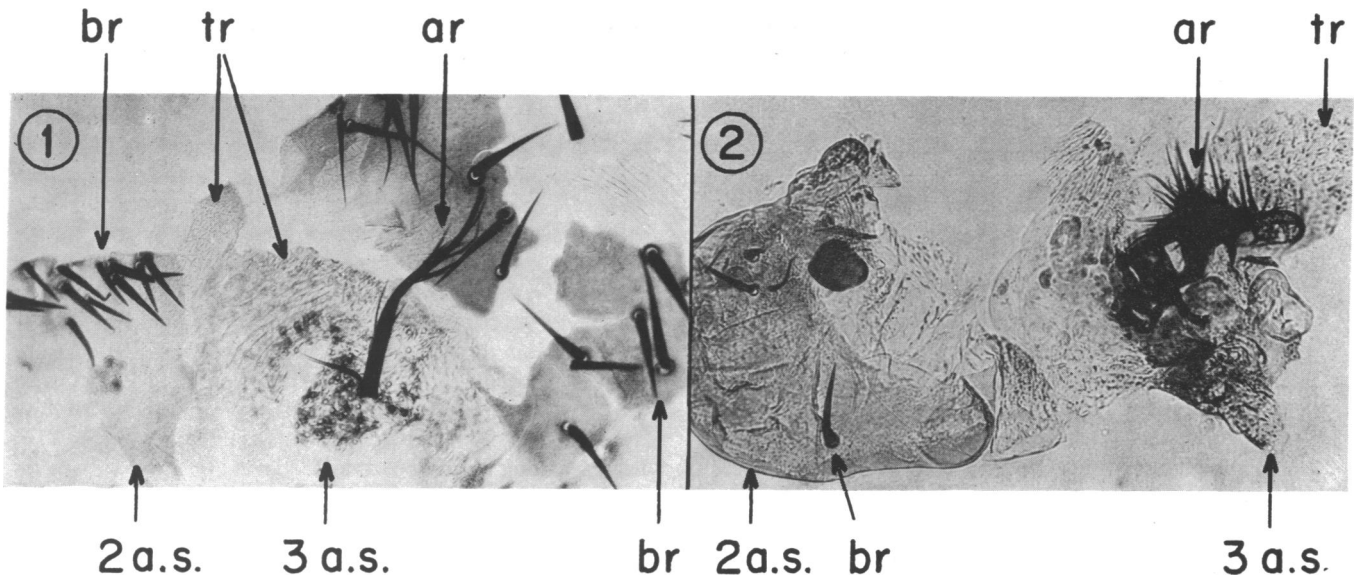

FIG. 4. An example of a developmentally abnormal antenna disc. The results of developmental tests (see *Materials and Methods*) of an antenna disc from a normal larva (panel *1*) and a class *D* mutant larva (panel *2*) are shown. The following antennal structures are identified by the arrows: *ar* arista, *br* large bristles, *tr* trichomes (hairs), *2 a.s.* second antennal segment, *3 a.s.* third antennal segment. The two major defects to be noted in the mutant are in the arista, which is morphologically abnormal, and in the large bristles, most of which are missing. Numerous trichomes are present in both the normal and mutant preparations. The magnification was ×200.

Class G—all discs are present and morphologically normal but some are developmentally abnormal

The abnormal discs in these mutants, as in the class *D* mutants, were distinguishable from the developmentally blocked discs by their capacity to form some of the characteristic adult structures (Fig. 4). One pair of discs was abnormal in seven of the mutants in this class, and two or more pairs of discs were abnormal in the other 19 mutants. All of the discs tested were represented among the abnormal discs, although not with equal frequencies (Table 3).

Class H—homeotic transformations

One pair of discs in each of the three mutants in this class had a homeotic abnormality that resulted in the formation of adult structures normally derived from another disc. In one mutant the antenna discs formed all the usual antennal structures except the arista, which was replaced by tarsal leg structures: this is the same transformation as in the viable homeotic mutant *aristapedia* (7). In a second mutant the haltere disc formed wing blade structures, a transformation that also occurs in the viable homeotic mutants *postbithorax* and *bithorax* (7). The third mutant had two homeotic transformations, one identical to the transformation in the preceding mutant (haltere disc forming wing blade structures) and another not previously described: The genital disc (male or female) formed only one of the normal structures (the anal plate), all of the other genital structures being replaced by tarsal leg and antennal structures.

DISCUSSION

The third-chromosome late-lethal mutants of *Drosophila* have proved to be a rich source of genetic defects causing abnormal development of imaginal discs. In nearly half of the mutants examined, one or more pairs of discs either were not present in the third-instar larvae or could not differentiate normally when tested by injection into normal host larvae. The frequency of disc abnormalities among these mutants probably is still higher, since the smaller discs were not examined.

Many of the genes controlling disc development, particularly those required for determination of the imaginal cells, must function before the pupal stage. We were concerned at first that this important class of early functioning genes might not be detectable using late lethal mutants, because of the possibility that a block in an early stage of disc development might prevent complete larval development. It is now evident that the discs are dispensable structures during the larval stage, as shown by the occurrence of the discless class *A* mutants. In view of the viability of a discless larva, probaby most, if not all, disc-specific abnormalities are compatible with larval viability. Thus, the late lethals should provide the mutants needed for a comprehensive analysis of the genetic control of disc development.

There are several ways in which a disc can be affected in the late lethal mutants. The most extreme disc abnormalities occur in the mutants that are missing discs (classes *A*, *B*, and *E*) or have morphologically and developmentally defective discs (classes *B*, *C*, and *F*). These abnormalities affect an entire disc, blocking either its formation or its capacity to differentiate. Another type of abnormality only partly affects development of a disc, which usually appears morphologically normal but produces both abnormal and normal adult structures (classes *D* and *G*). There are also homeotic transformations that result in a disc producing some of the structures normally derived from a different disc (class *H*). In each of the mutant classes except *B*, all of the affected discs in a mutant show the same type of abnormality. The uniformity of the disc phenotypes suggests that the abnormalities in each mutant are caused by a single genetic defect, and the results of genetic mapping and complementation experiments generally support this conclusion (Shearn and Garen, in preparation).

TABLE 3. *Developmentally abnormal discs in class* G *mutants*

Abnormal disc	Occurrence in mutants with one disc abnormal	Occurrence in mutants with two or more discs abnormal
Eye	0	10
Antenna	3	13
Wing	0	4
1st leg	3	17
2nd leg	0	15
3rd leg	1	17
Haltere	0	4

A total of 26 class *G* mutants were examined, of which seven had one pair of abnormal discs and the other 19 had two or more pairs of abnormal discs. All of the discs found to be abnormal are included in the table.

The class *B* mutants are exceptional in having two types of abnormalities: Some discs are missing, and the others are small and developmentally blocked. It is unlikely that this phenotypic heterogeneity is caused by multiple mutations, since the heterogeneous disc phenotpye of each mutant segregates as a unit in genetic crosses (Shearn and Garen, in preparation). The distinction between whether a disc is missing, or a small and developmentally blocked disc is formed, may be quantitative rather than qualitative.

There are extensive developmental interrelationships among the various discs. Many steps in disc development are common to all discs, as shown by the 29 mutants in which all discs are affected by the lethal mutation (classes *A*, *B*, *C*, and *D*); most of these mutants are in separate genetic complementation groups (Shearn and Garen, in preparation). In 24 other mutants (from classes *E*, *F*, *G*, and *H*) some but not all discs are affected, usually as a result of a single genetic defect. Many different combinations of abnormal discs occur among these mutants, but in all cases both members of a disc pair behave identically. The three pairs of leg discs generally are affected in the same way in a mutant, as are the eye and antenna discs, suggesting that the developmental pathways for the leg discs, and the eye and antenna discs, are closely linked. There also are specific developmental steps for individual disc pairs, as shown by the 10 mutants in which only one of the disc pairs is affected. One of these is a class *F* mutant with developmentally blocked antenna discs, seven are class *G* mutants (see Table 3), and two are class *H* mutants with a homeotic transformation in one disc pair.

The defective genes in the late lethal mutants could normally function at any stage of development between fertilization of the egg and emergence of the adult fly, a span of about 10 days. In the mutants with missing discs, development must be affected before the third larval instar when the discs normally become detectable by the procedure used in these studies. There are many *Drosophila* genes that control these early steps essential for disc formation, as indicated by the results of genetic complementation tests; among the 16 *A* and *B* mutants, only one pair failed to complement (Shearn and Garen, in preparation). The inability of these mutants to produce discs could occur for various reasons. For example, a defect in imaginal cell determination could block the formation of the progenitor cells for the discs; alternatively, a growth defect in the imaginal cells could prevent the discs from reaching a visible size in the larva, or a cell surface defect could prevent the imaginal cells from aggregating into stable disc structures. The analysis now in progress of temperature-sensitive lethal mutants with disc abnormalities should provide more precise information about the stages of development affected in the various mutant classes.

We express our appreciation to Mrs. Ruth Beaud, Mrs. Helen Ellis, Mrs. Frances Rice, and Mrs. Clara Sampson for their capable technical assistance, and to the National Science Foundation for financial support. T. R. was a predoctoral trainee of the National Institutes of Health, A. S. was a fellow of the Helen Hay Whitney Foundation, and W. G. was a fellow of the Jane Coffin Childs Memorial Fund.

1. Gehring, W., and R. Nöthiger, in *Developmental Systems: Insects*, ed. S. Counce–Nicklas, and C. H. Waddington (Academic Press, London, in press).
2. Beadle, G., and B. Ephrussi, *Genetics*, **21**, 225 (1936).
3. Mandaron, P., *Develop. Biol.*, **22**, 298 (1970). Gottschewski, G. H. M., *Wilhelm Roux Arch. Entwicklungsmech. Organismen*, **152**, 204 (1960). Schneider, I., *J. Exp. Zool.*, **156**, 91 (1964). Schneider, I., *J. Embryol. Exp. Morphol.*, **15**, 271 (1966).
4. Hadorn, E., *Developmental Genetics and Lethal Factors* (John Wiley, New York, 1961).
5. Lewis, E. B., and F. Bacher, *Drosophila Information Service*, **43**, 193 (1968).
6. Carlson, E. A., R. Sederoff, and M. Cogan, *Genetics*, **55**, 295 (1967).
7. Lindsley, D. L., and E. H. Grell, *Genetic Variations of Drosophila melanogaster* (Carnegie Inst. of Wash. Pub. No. 627, 1967).
8. Hadorn, E., G. Bertani, and J. Gallera, *Wilhelm Roux Arch. Entwicklungsmech. Organismen*, **144**, 31 (1949).