

Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*

I. Zygotic loci on the second chromosome

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1 Summary. In a search for embryonic lethal mutants on the second chromosome of *Drosophila melanogaster*, 5764 balanced lines isogenic for an ethyl methane sulfonate (EMS)-treated *cn bw sp* chromosome were established. Of these lines, 4217 carried one or more newly induced lethal mutations corresponding to a total of 7600 lethal hits. Eggs were collected from lethal-bearing lines and unhatched embryos from the lines in which 25% or more of the embryos did not hatch (2843 lines) were dechorionated, fixed, cleared and scored under the compound microscope for abnormalities of the larval cuticle. A total of 272 mutants were isolated with phenotypes unequivocally distinguishable from wild-type embryos on the basis of the cuticular pattern. In complementation tests performed between mutants with similar phenotype, 48 loci were identified by more than one allele, the average being 5.4 alleles per locus. Complementation of all other mutants was shown by 13 mutants. Members of the complementation groups were mapped by recombination analysis. No clustering of loci with similar phenotypes was apparent. From the distribution of the allele frequencies and the rate of discovery of new loci, it was estimated that the 61 loci represent the majority of embryonic lethal loci on the second chromosome yielding phenotypes recognizable in the larval cuticle.

Key words: *Drosophila* – Larval cuticle – Pattern formation – Embryonic lethal mutations

Introduction

In the development of higher organisms, an apparently uniform egg cell gives rise to a complex, strictly organized pattern of differentiated tissue. The principles governing the correct segregation of developmental potential in a defined spatial and temporal order are poorly understood. We are studying this process in *Drosophila melanogaster*, using mutations as tools to alter or block developmental decisions during embryogenesis. Although *Drosophila* mutants have been collected since the beginning of this century, systematic searches for mutations affecting embryonic de-

velopment have only begun recently and the few described mutants showing a distinct embryonic phenotype had been isolated more or less by chance (for review, see Wright 1970a). For an understanding of the logic behind the process of embryonic differentiation and determination, as well as for estimating the complexity of the system and the types of decisions in early development, it is necessary to know how many genes are involved in embryonic pattern formation, whether each of these genes is unique and what types of pattern alterations can be caused by mutation in a single gene. To this end, we have begun **large-scale mutagenesis experiments** designed to identify all genes required for the development of a larva with normal external morphology.

By their mode of inheritance, two types of genes affecting embryonic development may be distinguished: genes whose activity is required during **oogenesis** (maternal effect genes) and genes whose activity is required **after fertilization** in the zygotic genome (zygotic genes). Systematic searches for maternal effect mutants have been carried out in several laboratories recently (Rice 1973; Gans et al. 1975; Mohler 1977), and a few maternal effect mutants were described with respect to their effect on the embryonic pattern (Rice and Garen 1975; Zalokar et al. 1975; Nüsslein-Volhard 1977a, 1979). The phenotypes of the few maternal effect mutants which have been studied in some detail suggest that the maternal contribution to embryonic pattern formation is of a rather general and global nature, defining the spatial coordinates of the developing embryo (Nüsslein-Volhard 1979). This indicates that much of the information for spatial diversification and differentiation is provided by the zygotic genome.

Very few mutagenesis screens have been carried out for zygotic embryonic mutations, since, compared to mutations affecting the morphology of the adult fly, those that affect the morphology of the larva appeared in the past difficult to obtain, recognize and analyse. The few well-studied mutations with an effect on larval morphology have been primarily detected by other criteria, e.g. a dominant phenotype visible in adult flies [*Ubx* (Lewis 1978), *Antp* (Wakimoto and Kaufman 1981), *Notch* (Poulson 1940), *Krüppel* (Gloor 1950)] or a recessive visible adult phenotype in weak alleles of the respective loci [*en* (Kornberg 1981)]. The embryonic phenotypes of these mutants support the notion that it is the zygotic class of genes which is involved in the more specific embryonic decisions and the interpretation of the maternally provided positional information. Assuming that mutations in such genes would lead to morphological ab-

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normalities in the mutant embryo and to a failure to hatch from the egg case, we set out to isolate embryonic lethal mutants with a phenotype visible in the lethal individuals (embryonic visible mutations).

In a saturation screen for embryonic visible mutations several difficulties are encountered. The *Drosophila* genome contains about 5000 genes, 90% of which can mutate to lethality. Approximately one-third of these are embryonic lethals (Hadorn 1955). It is not known a priori what fraction of these show a visible lethal phenotype. Since there is no selective system for lethal mutations, a very large number of lines inbred for a mutagenized chromosome must be established and the lethal individuals inspected microscopically for morphological phenotypes. In the experiment described in this paper, we made use of three recently developed techniques which facilitated the work required for a screen aimed at saturation. We used dominant temperature-sensitive mutations (Suzuki 1970) for killing unwanted progeny at the restrictive temperature, thus avoiding the time-consuming sorting of flies during the inbreeding generations (Wright 1970b). The block-agar method (Nüsslein-Volhard 1977b) allowed eggs to be collected from large numbers of lines simultaneously. A whole mount technique for cuticular preparations (Van der Meer 1977) was used to detect even subtle abnormalities in the lethal embryos.

In this paper we describe the isolation of 272 embryonic visible mutants which define 61 complementation groups on the second chromosome of *D. melanogaster*. A brief account of the screen has been published elsewhere (Nüsslein-Volhard and Wieschaus 1980). Similar screens for mutants on the first and third chromosome will be described in the accompanying papers (Jürgens et al. 1984; Wieschaus et al. 1984a).

4 Materials and methods

5 Strains. For mutagenesis, a *cn bw sp* chromosome was used which had been previously isogenized and shown to be lethal-free. *DTS91* is one of the dominant temperature-sensitive mutants mapping in a cluster near *cl* (Suzuki and Procurier 1969; Wright, personal communication). The temperature-sensitive period of heterozygous *DTS91* individuals lies at the end of embryogenesis (unpublished work).

6 The *DTS91* chromosome was further marked with the recessive markers *b*, *pr*, *cn* and *sca* for the easy detection of escapers which had survived the temperature treatment. *DTS91 b pr cn sca/CyO* flies have an orange eye-colour compared to the bright red eye-colour found in *cn bw sp/CyO* flies. Throughout the experiments, *CyO* (*In (2LR) O*, *dp^{l1}Cy pr cn²*) was used as a balancer. Homozygous *CyO/CyO* individuals die in larval stages. For the mapping, in addition to the recessive markers *cn* (57), *bw* (104) and *sp* (107) present on the mutant chromosome, a chromosome carrying the dominant markers *S(1)*, *Sp(22)*, and *Bl(55)* was used. Some of the mutants were further mapped using an *al dp b pr vg^{np}* chromosome and back-crossing with a *b pr cn vg^P* chromosome.

The *engrailed* and *wingless* alleles were identified in complementation tests with *en⁴*, a lethal *engrailed* allele provided by T. Kornberg, San Francisco, Cal. USA, and with *wg^{L3}*, a lethal allele of *wingless* (Babu 1977). W. Gelbart, Cambridge, Mass. USA provided *dpp²⁷*, an embryonic lethal allele of *decapentaplegic*. The original *Kr* allele was

obtained from H. Gloor, Geneva, Switzerland, USA and representative alleles of the lethal complementation groups *l(2)br27* and *l(2)br28* from M. Ashburner, Cambridge, England. The wild-type stock was Oregon R. Deficiency stocks and marker mutants were obtained from the *Drosophila* stock centres at Caltech, Pasadena, Cal. USA or Bowling Green, Ohio, USA or directly from the discoverer (see Table 1). The description of marker mutants and deficiencies may be found in Lindsley and Grell (1968). If not otherwise indicated, flies were grown at 18° or 25° C in humidified rooms.

Flyfood. Flies were grown on standard medium, containing (per litre of water) 8 g agar, 18 g dried yeast, 10 g soybean meal, 7 g molasses, 80 g malt extract, 80 g cornmeal and 6.3 ml propionic acid. Eggs were collected on apple juice-agar plates: 1 l apple juice, 3 l water, 95 g agar, 100 g sugar and 40 ml 15% Nipagin in 95% ethanol.

Establishment of lethal lines. A total of 1500 *cn bw sp* males were fed with 25 mM ethyl methane sulfonate (EMS) in 1% sucrose according to the method of Lewis and Bacher (1968), with the modification that they were starved (on Kleenex saturated with water) for 4 h prior to the EMS treatment. They were mated with 2500 *DTS91 b pr cn sca/CyO* virgin females. Progeny were raised at 22° C. The male parents were discarded after 5 days. Single F1 males of either the genotype *cn bw sp/CyO* or *cn bw sp/DTS91 b pr cn sca* were mated with one or two *DTS91 b pr cn sca/CyO* virgin females. These matings were set at 29° C for the first 3 days, after which the parents were discarded with help of a vacuum cleaner. Drops of a yeast suspension were added to each tube to prevent desiccation and allow optimal growth of progeny. After 1 further day at 29° C, the tubes were transferred to a lower temperature. The F2 flies (of the genotype *(cn bw sp)*/CyO*) were transferred to fresh tubes and an F3 generation raised. Except for the first 4 days after setting up the single male matings at 29° C, the temperature was chosen between 18° and 25° C such that the F3-generation flies emerged within a period of 2 weeks. The F3 progeny was scored for the absence of white-eyed, straight-winged flies (*cn bw sp* homozygotes). Lines which did not yield enough progeny (less than 5% of the F2 tubes) were grown for one further generation. The crossing scheme is described in Fig. 1.

Screening of embryos. Eggs were collected from lethal-bearing lines using the block method (Nüsslein-Volhard 1977b), modified to allow the collection of eggs from several females per tube. Blocks were made from 18 plastic test tubes (14 × 120 mm), glued together into a pattern of four rows (5 + 4 + 5 + 4 tubes). Flies from each line were shaken into one tube with the help of a small funnel. The number of flies per tube was approximately 10–30. It was adjusted by setting up the F3 tubes at a time at which only approximately one-third of the flies had hatched. Eggs were collected by inverting the blocks over apple juice-agar plates supplemented with fresh yeast suspension, in two 24-h periods at 25° C. The first egg collection was discarded. After removal of the flies, the second plates were supplemented with fresh yeast suspension, distributed around the edge of the plates in order to trap hatched larvae. After 36 h of development at 25° C, the fraction of unhatched eggs was estimated for each line and the unhatched eggs from

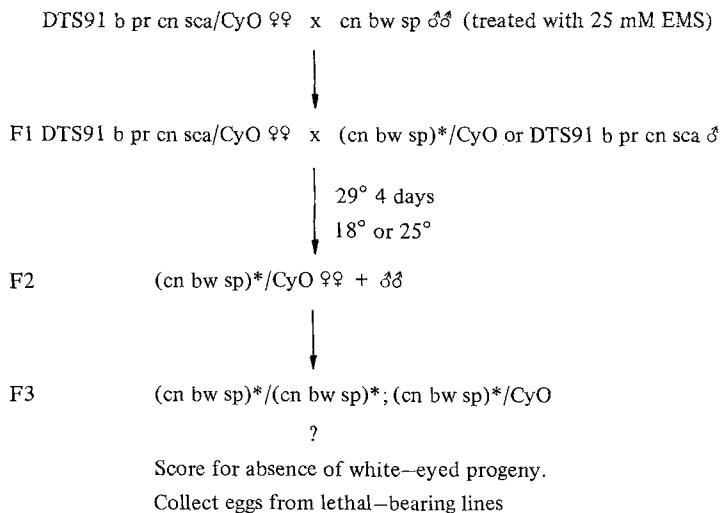


Fig. 1. Crossing scheme for establishing lines isogenic for an individual ethyl methane sulfonate (EMS)-treated *cn bw sp* chromosome

lays containing more than 20% unhatched eggs collected. For dechorionation and fixation, the eggs were transferred into small sieves made by melting rings cut from polyethylene tubing (6 mm diameter) on a stainless steel net. Each sieve had seven rings, allowing processing of eggs from seven strains simultaneously. The sieves were first placed in 0.7% NaCl, 0.02% Triton X 100 solution for washing, then in a 50% dilution of commercial bleach (12% sodium hypochlorite) for dechorionation and finally in glycerol-acetic acid (1:4) for fixation. Between each step, the excess liquid was blotted off on a paper towel. Fixation was performed at 60° C for 1 h. The fixed eggs were transferred from the screens with a fine brush into a small drop of Hoyer's medium (30 g gum arabic, 50 ml H₂O, 200 g chloral hydrate, 20 g glycerol) and covered with a 10 × 10 mm cover-slip. Eggs from one screen (seven lines) were mounted onto one slide. The eggs were cleared by overnight incubation at 60° C. They were examined by two observers simultaneously on a Zeiss discussion microscope at 400 times magnification (Zeiss Neofluar 40 × Ph).

Recovery and confirmation of mutant lines. Lines of interest were recovered from the original tube which had been kept at 18° C during the egg-screening procedure. The flies were transferred to fresh tubes and allowed to lay eggs for several days in order to maintain the stock, then transferred into egg-collecting blocks. The egg-screening procedure was repeated; however, in this second screen the hatch rates were determined more accurately by counting the number of unhatched eggs per 100 total eggs. Confirmed mutants were isogenized a second time using the *DTS91* procedure outlined above. Each line was carefully checked for the absence of *DTS91* escapers and, if necessary, purified by collecting virgin females in this generation. Tests for the presence of the mutant phenotype in the isogenized lines were usually performed by inspecting living embryos in transmitted light under oil (Voltalef 3S or paraffin oil).

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Recovery of third-chromosomal mutants. Lines which contained putative embryonic lethal mutations apparently mapping on the third chromosome were tested by collecting eggs from 20–40 individual females mated to sibling males. The progeny of females producing the mutant phenotype were raised and five males from the next generation crossed to virgin females carrying the *TM3* balancer chromosome.

Ten lines isogenic for one third chromosome from the original stock were established by crossing *TM3* males individually to *DTS7/TM3* (Holden and Suzuki 1973) virgin females. Except for the first few days, the crosses were performed at 29° C such that all progeny carrying the *DTS7* chromosome died. The lines were tested for the presence of the mutation by inspecting living embryos.

Test for putative translocations and semi-dominant maternal effects. In order to test whether or not the embryonic phenotype was ascribable to recessive zygotic lethality, heterozygous mutant females were crossed with wild-type (Oregon R) males and the eggs from these crosses inspected for the presence of the mutant phenotype. If the phenotype occurred in these crosses, it was probably caused either by a semi-dominant maternal effect mutation or by a translocation. In order to distinguish between the two alternatives, the reciprocal cross (wild-type females × mutant heterozygous males) was also performed. These tests were performed only on a small number of lines, which behaved unusually in other tests.

Complementation. Crosses were performed between mutants with similar phenotype. Eggs were collected from three pairs of flies using the block system and unhatched eggs inspected under oil. In doubtful cases, the parents were recovered from the egg-laying tubes and progeny raised. They were checked for the viability of **transheterozygotes**. In certain phenotypic classes in which the embryonic phenotype could not easily be identified in living embryos, non-complementation for lethality was checked first, followed by analysis of the embryonic phenotype in transheterozygotes.

Recombination mapping. One or two members of each complementation group were mapped by recombination with the markers *S*, *Sp*, *Bl*, *cn* and *bw*. Females heterozygous for the mutant-bearing *cn bw sp* chromosome and an *S Sp Bl* chromosome were back-crossed to (a) males heterozygous for the *cn bw sp* chromosome carrying an independent allele to the mutant to be mapped and the *CyO* chromosome, and (b) to *cn bw sp* homozygous males. Non-*Cy* progeny from cross (a) were scored for the frequency of the markers from which the approximate map position was calculated. Suitable recombinant males from cross (b) were

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Table 1. Deficiencies and translocations on the second chromosome

	Deficiency breakpoints	Phenotype homozygous	Uncovers	Reference
T(1; 2)odd ^{1,10}	21A; 24B	—	odd	a
T(Y; 2)odd ^{4,25}	21A; 24D	—	odd, slp	a
T(Y; 2)odd ^{2,31}	21A; 25C	—	odd, slp	a
Df(2L)al	21B8-C1; 21C8-D1	“u-shaped”	ush	b
Df(2L)S ²	21C6-D1; 22A6-B1	“Star”, head	S, lea	b
Df(2L)S ³	21D2-3; 21F2-22A1	“Star”	S	b
T(Y; 2)odd ^{4,13}	22A; 25F	—	odd, mid, slp	a
T(2; 3)odd ^{5,1}	23E; 24E	—	odd, slp	a
Df(2L)M-z ^B	24E2-F1; 25A1-2	normal	mid	b
Df(2L)cl.1	25D7; 25F1,2	“midline”, head	mid	c
Df(2L)cl.7	25D7; 26A7,8	“midline”,	mid	c
Df(2L)GdhA	25D7-E1; 26A8-9	“midline”, head	mid	p
Df(2L)J-der27	31B; 31E	poorly differentiated cuticle and head	pim, bsk	a
T(2; 3)prd ^{2,27}	31B; 33DE	—	prd, bsk	a
Df(2L)Prl	32F1,2; 33F1,2	“paired” (faint)	prd, sal	d
T(Y; 2)prd ^{5,12}	33A; 35B	—	prd, sal	a
Df(2L)prd ^{1,7}	33B3-7; 34A1,2	“paired” (faint)	prd	a
Df(2L)prd ^{1,25}	33C1,2; 33E1,2	—	prd	a
Df(2L)b75	34D4-6; 34E5-6	normal	ck	c
Df(2L)64j	34E5-F1; 35C3-D1	“crinkled” (faint)	ck	b
Df(2L)A260	35B1-2	normal	sna, ck	e
Df(2L)75c	35A1,2; 35D4-7	“snail”	c	a
DF(2L)H20	36A8-9; 36E3,4	normal	f	
Df(2L)H68	36B1,2; 37A1,2	normal		
Df(2L)137	36C2-4; 37B9-C1,2	poorly differentiated, tail up	g	
Df(2L)M-H ^{ss5}	36D1-E1; 36F-37A	poorly differentiated	g	
Df(2L)50	36E4-F1; 38A6,7	holes in cuticle, head abnormal	spi, IG76, Ddc	g
Df(2L)158	37B2-8; 37E2-F4	unpigmented, normal morphology	Ddc	g
Df(2L)E55	37D2-E1; 37F5-38A1	“spitz”	spi	g
Df(2L)2	37D2-E1; 38E6-9	holes in cuticle, head abnormal	spi, IG76	g
Df(2L)9	37E2-F4; 38A6-C1	holes in cuticle, head abnormal	spi, IG76	g
Df(2L)84	37F5-38A1; 39D3-E1	holes in cuticle, head abnormal	IG76	g
Df(2R)pk ^{78s}	42C1-7; 43F5-8	poorly differentiated	h	
Df(2R)42	42C3-8; 42D2-3	normal	b	
Df(2R)pk ^{78k}	42E3; 43C3	poorly differentiated	h	
Df(2R)ST1	43B3-5; 43E18	—	h	
T(2; 3)eve ^{1,18}	44B; 46DE	—	ptc, flz, eve, lin	a
T(2; 3)eve ^{2,28}	44F; 47A	—	flz, eve, lin	a
Df(2R)eve ^{1,27}	46C3-4; 46C9-11	unsegmented	eve	a
Df(2R)en ^A	47D3; 48B4,5	segments fused, poorly differentiated	en, shn, sha	i
Df(2R)en ^B	47E3-6; 48B2	segments fused, poorly differentiated	en, shn, sha	i
Df(2R)vg ^C	49B2-3; 49E2-5	normal	b	
Df(2R)vg ^D	49C1-2; 49E7-F1	head broad	Psc	b
Df(2R)vg ^B	49D3-4; 50A2-3	undifferentiated	Psc	b
Df(2R)L ⁺⁴⁸	51A1; 51B4	head broad	Asx	k
Df(2R)PC4	55A; 55F	poorly differentiated	thr, IM45	l
Df(2R)bw ⁵	59D10-E1; 59E4-F1	normal	b	
T(1; 2)Bld	60B12-13; 60F5	—	Kr, gsb, zip	b
Df(2R)Px ²	60C5-6; 60D9-10	normal	b	
Df(2R)M-c ^{3,3a}	60E2-3; 60E11-12	poorly differentiated	b	
T(1; 2)sc ⁸²	60E6-8; 60F5	—	Kr, gsb, zip	b, l
Df(2R)IIX62	60E9-10; 60F1-2	“gooseberry”	gsb, zip	m
Df(2R)SBl	60F1; 60F5	“Kr, gsb”	Kr, gsb	n
T(1; 2)B80)	60F2,3; 60F5	“Krüppel”	Kr	o

^a Nüsslein-Volhard, unpublished work; ^b Lindsley and Grell 1968; ^c Ashburner et al. 1980; ^d Baker, personal communication; ^e Ashburner et al. 1982; ^f Wieschaus, unpublished work; ^g Wright et al. 1976b; ^h Ashburner et al. 1981; ⁱ Gubb, personal communication; ^k McIntyre, personal communication; ^l Jürgens, personal communication; ^m this paper; ⁿ Jäckle, personal communication; ^o Lindsley et al. 1972; ^p Grell 1967

collected, individually mated to virgin females of an allelic mutant stock and the progeny from these crosses inspected for the mutant phenotype. For some of the complementation groups only cross (a), mapping of the lethal function, and in others only cross (b), mapping of the mutant phenotype, was made. Map positions based on lethality were cal-

culated by counting at least 150 progeny. The number of recombinant males tested for the mutant phenotype varied between 20 and 200, depending on the distance between flanking markers. Some mutants mapping close to the centromere were further mapped using the recessive markers *b*, *pr*, *cn* and *vg*. Females heterozygous for *al dp b pr vg^{np}*

and the mutant-bearing *cn bw sp* chromosome were back-crossed with *b pr cn vg^D* males. Recombinant males with all possible marker combinations were tested for the presence of the mutant allele as outlined above (cross b).

18 Cytological localization. Complementation tests were performed with chromosomes carrying a deficiency in the region close to the map position of the respective mutant. The deficiencies used in the present study are listed in Table 1.

Results

19 Mutant screen

The crossing scheme for the establishment of lethal-bearing lines is illustrated in Fig. 1. The scheme makes use of the dominant lethality at high temperature associated with *DTS91*, which obviates the necessity to collect virgin females from each line in the F2 generation. Lethal-bearing lines were recognized in the F3 generation by the absence of white-eyed, non-Cy (*cn bw sp* homozygous) progeny.

Of approximately 10000 F1 crosses set up, 5764 yielded enough progeny to be successfully tested for embryonic lethality. About 5% of these yielded too few progeny in the F3 generation and were tested only in the F4 generation. Inspection of the tubes after emergence of about one-third of the flies yielded 4580 lines without white-eyed progeny. Some of these lines were checked again after all flies had emerged and 8% of them were found to eventually produce some white-eyed progeny. Thus, the total number of lethal lines was about 4217 or 73%, corresponding to 1.3 lethal hits per chromosome.

Eggs were collected from the 4580 lines. In lines bearing an embryonic lethal mutation on the second chromosome, 25% of the eggs were expected not to hatch, while in lines with larval or pupal lethals all eggs were expected to hatch. However, a clear-cut distinction between embryonic and later lethals was not possible on the basis of the hatch rate, owing to a rather high and erratic background of unhatched eggs in almost all lines. Therefore, the unhatched eggs from all lines producing more than 20% unhatched eggs (2843 lines) were processed for microscopic inspection.

The whole mount technique used (van der Meer 1977) leads to a dissolution and clearing of all soft parts of the embryos (larvae), while chitinized and sclerotinized structures are revealed in very good detail (Lohs-Schardin et al. 1979). In the screen of the cuticular preparations of the lines, which was always done by two observers simultaneously, the lines were first distributed into four large categories. This classification was based on the developmental performance of the unhatched eggs, compared to the estimated hatch rates.

1. Embryonic viable. Most of the unhatched eggs were undeveloped (probably unfertilized). Developed embryos were significantly less than 25% of the original lay, and they often showed heterogeneous, unspecific defects.

2. Embryonic lethal with normal morphology. Most of the eggs developed to the unhatched larval stage. No morphological abnormality could be detected in the cuticle even at high magnification.

3. Embryonic lethal, poorly differentiated. Most of the eggs developed, but did not reach the stage of complete differentiation of the larval cuticle. The phenotype in embryos of this class was clearly distinguishable from normal embryos. Often the cuticle was not fully pigmented, or it had necrotic signs appearing as small pimples scattered over the surface. In several of these lines, the larvae turned on a deep brown pigmentation. Otherwise, the general morphology of the larval cuticle appeared normal.

4. Embryonic lethal with abnormal morphology. Among the unhatched embryos, a substantial fraction showed a clear-cut and specific phenotype. The deviations from normal morphology ranged from unpigmented cuticle with otherwise normal differentiation, through segmentation abnormalities, head defects, holes in large specific cuticular regions, abnormal arrangement of segments to apparent lack of cuticular structures. The detailed description of the phenotypes will be published later.

From the 2843 lines which were tested in the initial screen, 504 lines were kept and retested immediately. Five of these lines were lost before retesting. The 504 lines included all of the fourth category lines (mutants with phenotypes), those of all other categories in which a clear assignment to one of the four categories could not be made in the first screen, as well as several lines which possibly carried an interesting mutant on the third chromosome.

In the retest, the hatch rate was determined accurately and it was determined whether the number of mutant embryos corresponded to the 25% expected for a recessive lethal. In several cases, the mutant embryos were less frequent than expected. Some of these lines were found to be contaminated with *DTS91* escaper flies and the second-chromosomal mutants could be recovered by re-isogenization. The actual number of lines containing escapers was not counted, but did not exceed 10%–15%.

In other lines, the phenotype was apparently caused by a recessive mutation on the third chromosome, since only a small fraction of single-pair matings produced embryos with the respective mutant phenotype. In 24 of 28 cases, an isogenization of third chromosomes resulted in the isolation of mutants mapping on the third chromosome, including one recessive maternal effect mutant. From two lines, a second as well as a third chromosomal mutant was recovered, and in one case two third-chromosomal mutants were recovered from a single line.

Some of the lines showed phenotypes which were more variable and heterogeneous than those of the confirmed second-chromosomal mutants. Often the frequency of mutant embryos by far exceeded the expected 25%. These lines were tested for the presence of dominant lethality in reciprocal crosses with wild-type flies. In 31 of these lines, mutant phenotypes were produced if mutant females were mated with wild-type males, but not reciprocally, suggesting that these lines contained a leaky dominant maternal effect mutation. In 20 cases, mutant phenotypes were found in both types of matings. In these lines, the phenotype was probably caused by aneuploid segregants of translocations. In a few other cases, the phenotype was gradually lost in successive generations.

After retesting and a second isogenization, 321 lines were finally established and confirmed as second-chromosomal, embryonic lethal mutations causing a distinct pheno-

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Table 2. Screen for embryonic lethal mutants on the second chromosome

	<i>n</i>	% of lethal hits
Total lines tested	5764	
Lethal lines	4217	
Lethal hits ^a	7581	100
Embryonic lethal lines	1620	
Embryonic lethal hits ^a	1907	25
Phenotypes of embryonic lethal lines		
a) normal morphology	1021	17
b) poorly differentiated	282	3.7
c) subtle deviations from normal	45	0.6
d) synthetic	4	0.06
e) abnormal morphology	268 ^b	3.6

^a Calculated from the Poisson distribution

^b Four lines carry two mutations

type in cuticular preparations. The results of the screen are summarized in Table 2.

Complementation analysis

In order to determine the number of loci which were represented as mutant in the 321 mutants with embryonic phenotypes, crosses were performed between pairs of mutants. The occurrence of embryos showing the respective phenotype served as a criterion for allelism in most cases. In several instances, the absence of viable transheterozygotes in the adult progeny was also checked.

Since it was impossible to cross each mutant with all others, our strategy was to establish small complementation groups on the basis of similar phenotypes and then use only one or two members to test other candidates. To this end, the mutant phenotypes were classified in five broad categories, according to purely pragmatic criteria. These were: (1) anteroposterior pattern, (2) dorsoventral pattern, (3) holes in cuticle, (4) differentiation, (5) head defects.

Each category contained several smaller groups, established with the aim of predicting allelism as closely as possible on the basis of the phenotype. As new mutants were classified, they were checked to see whether they fit into already existing subgroups and, if this failed, a new subgroup was established. Several mutants were tested as members of more than one subgroup.

In general, this strategy proved useful and in many instances our predictions of allelism were correct. (Needless to say, our abilities in this matter improved greatly during the course of the experiment.) For example, all nine members of the "three row" group turned out to be allelic, and in the case of *wingless*, five of the six alleles were classified correctly, while the sixth because of its lower expressivity, was initially listed in another subgroup. Of the 14 members of the "faint little ball" group 12 were allelic, while 2 turned out to be translocations. In several instances, members of one group made up two or more complementation groups. Examples are the "dorsal open" group containing two loci, with 17 and 2 alleles, or the "unpigmented cuticle" group which formed three loci with 6, 4 and 3 alleles each.

In other cases, the initial classification was less successful. Several mutant phenotypes had more than one prominent feature and what later turned out to be allelic mutants had initially been distributed among two or three different categories. For example, the nine alleles of *ribbon* were initially listed under "shaven II" "faint

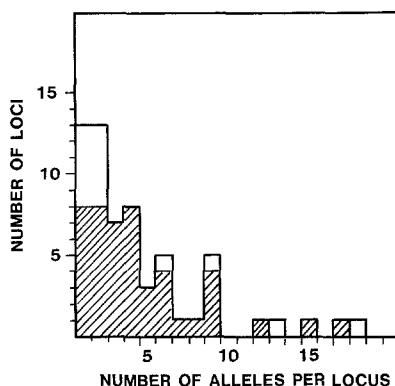


Fig. 2. Distribution of allele frequencies. The *upper line* indicates the number of loci with a given number of alleles for all 61 loci (272 mutants). The *shaded area* gives the distribution of allele frequencies excluding head mutants (47 loci, 211 mutants)

dorsal closure" and "small belt". The classification system was also less successful for loci, alleles of which showed different expressivity of the mutant phenotype. In *shotgun* (18 alleles), for example, the 7 strong alleles were classified in one group, while 11 alleles with lower expressivity were initially listed under "ventral open", "ventral holes" and "head open". Thus, in later periods of this work, the number of established complementation groups, which had initially increased fast, declined again since several separately established complementation groups turned out to be allelic to other groups.

While the attribution to potential complementation groups on the basis of the phenotypes presented no severe problems for most mutants, two phenotypic categories proved very difficult. Mutants of the first category (approximately 40 mutants) showed rather subtle deviations from normal; they were initially classified under "denticle morphology", "head skeleton differentiation" or "narrow denticle belts", often overlapping two or even three groups. We failed to establish complementation groups for most of these mutants. Upon reinspection, we noticed that, although the mutants all had a distinct phenotype, this phenotype came very close to wild-type or to mutants of the "poorly differentiated" class which we had discarded. One possibility explaining our failure to establish allelism among these mutants is that we had not always recognized the phenotype in the initial screen. Furthermore, the phenotype might have been caused by an additive effect of two or more independent mutants on the same chromosome, none of which caused a significant phenotype by itself. The mutants of this class were excluded from all further analysis.

Another difficult class of phenotypes was the one in which mutant embryos showed head defects as the most prominent phenotypic feature. Of the mutants, 70 belonged to this class and, following the strategy outlined above, only one-third of them could be assigned to complementation groups. Furthermore, prediction of allelism was incorrect more frequently than in the other phenotypic classes. Therefore, for this phenotypic class a complete complementation matrix was set up and 59 mutants crossed to each other. These mutants included the 44 "left overs", one representative allele of each of the eight previously established complementation groups with head phenotypes, and of seven other complementation groups which, on the basis of their phenotypes, were likely to not complement some of

Table 3. Loci with an embryonic visible phenotype on the second chromosome

Locus	Phenotype	Number of alleles			Map position ^a	Cytology ^b
		total	weak	ts		
<i>Additional sex combs (Asx)</i>	head broad, homoeotic, head and thorax partially transformed into abdomen	1	—	—	72	51A1-51B4
<i>anterior open (aop)</i>	head open	6	—	—	(12)	
<i>arrow (arr)</i>	denticle belts broad, pointed	9	—	—	66	
<i>basket (bsk)</i>	dorsal anterior hole	3	—	—	33	31B-31E
<i>big brain (bib)</i>	ventral cuticle absent, hypertrophy of central nervous system	5	—	—	32	
<i>broad head (bhe)</i>	head broad	9	—	—	(0)	
<i>brown head (brh)</i>	head broad	7	—	—	61	
<i>clift (cli)</i>	head broad	2	—	—	17	
<i>crack (cra)</i>	head broad	2	—	—	77	
<i>crinkled (ck)</i>	denticles thickset and forked, hairs basally fused, short; sensory hairs blunt. Adult escapers short bristles, basally fused and short trichomes	4	1	—	51	35B2-35B10 ^g
<i>decapentaplegic (dpp)</i>	embryo twisted, denticle bands spread laterally, gastrulation abnormal	1	1	—	4	22F2 ^c
<i>Dopadecarboxylase (Ddc)</i>	unpigmented cuticle and mouth parts	3	1	—	54.0	37C1,2 ^d
<i>engrailed (en)</i>	anterior margin of each segment defective, pair rule defects in naked cuticle of T1, T3, A2, A4, A6, A8	6	2	1	62	48A2 ^e
<i>even-skipped (eve)</i>	pair rule segmentation defects, denticle band and adjacent cuticle of T1, T3, A2, A4, A6, A8 deleted	2	1	1	59	46C3-11
<i>faint (fai)</i>	unpigmented cuticle and mouth parts, larval lethal	6	2	—	61	
<i>faint little ball (flb)</i>	ball of dorsal cuticle, ventral hypoderm absent	15	2	3	101	
<i>faint sausage (fas)</i>	poorly differentiated cuticle and head	4	—	—	68	
<i>filzig (flz)</i>	denticle differentiation abnormal	5	—	—	59	(44F-46D)
<i>fizzy (fzy)</i>	ventral cuticle and CNS degenerated	8	—	—	51	
<i>ghost (gho)</i>	undifferentiated cuticle	3	—	—	68	
<i>gooseberry (gsb)</i>	segment polarity mutant, mirror image duplication of denticle bands in all segments	1	—	—	107.6	60E9-F1
<i>Krüppel (Kr)</i>	segments T1 through A5 deleted, A6 duplicated	3	3	—	107.6	60F2-5
<i>leak (lea)</i>	head broad	2	—	—	3	21F2-22B1
<i>lines (lin)</i>	small anterior portion of each segment deleted. A8, spiracles, and anal plates absent	2	—	—	59	(44F-46D)
<i>master mind (mam)</i>	ventral cuticle absent, hypertrophy of central nervous system	9	7	—	71	
<i>midline (mid)</i>	denticle bands defective in ventral midline	3	1	—	16	25E-25F1,2
<i>mummy (mmy)</i>	mouth parts and denticles poorly differentiated	5	1	1	(16)	
<i>odd-skipped (odd)</i>	pair-rule segmentation defects, partial deletion of denticle bands of T2, A1, A3, A5, A7	2	1	—	8	(23E-24B)
<i>paired (prd)</i>	pair-rule segmentation defects, deletion of denticle bands of T1, T3, A2, A4, A6, A8, and naked cuticle of T2, A1, A3, A5, A7	3	2	1	45	33B6,7-E2,3
<i>patched (ptc)</i>	segment polarity mutant, mirror image duplications of all segment boundaries	12	2	1	59	(44B-44F)
<i>pimples (pim)</i>	poorly differentiated cuticle and head	1	—	—	30	31B-31E
<i>Posterior sex combs (Psc)</i>	head broad, homoeotic, head and thorax partially transformed into abdomen	1	—	—	67	49E2-F1
<i>raw (raw)</i>	dorsal closure and cuticle differentiation defective	3	—	—	19	
<i>ribbon (rib)</i>	lateral extent of belts narrow, fusion of adjacent denticle bands in ventral midline, dorsal closure defective	9	2	—	88	
<i>scab (scb)</i>	small mid-dorsal hole	4	—	—	73	
<i>schlaff (slf)</i>	arrangement of cuticle abnormal, head skeleton tilted	6	—	—	15	
<i>schnurri (shn)</i>	dorsal hypoderm absent	17	7	—	62	47E3-48B2

Table 3. (continued)

Locus	Phenotype	Number of alleles			Map position ^a	Cytology ^b
		total	weak	ts		
<i>shavenoid (sha)</i>	denticles sparse, hairs short or absent. Viable in adults, trichomes absent	4	—	—	62	47E3-48B2
<i>shotgun (shg)</i>	many small holes in cuticle	18	11	—	92	
<i>slater (str)</i>	dorsal hypoderm absent	2	—	—	17	
<i>slit (sli)</i>	head broad	2	—	—	77	
<i>sloppy paired (slp)</i>	pair-rule segmentation defects, partial deletion of naked cuticle of T2, A1, A3, A5, A7	1	—	—	8	(24C-24D)
<i>smooth (smo)</i>	all denticles point posteriorly. At 18°, naked cuticle deleted, denticle bands enlarged	2	—	—	4	
<i>snail (sna)</i>	belts narrow, larva twisted, no ventral furrow in gastrulation	1	—	—	51	35C3-35D
<i>spalt (sal)</i>	head broad	2	—	—	44	32F1,2-33B
<i>spitz (spi)</i>	head skeleton pointed, deletion of median parts of all denticle bands	4	—	—	54	37E2-38A1
<i>Star (S)</i>	head skeleton pointed, deletion of median parts of denticle bands, dominant S-phenotype in adults	4	—	—	1.3	21D2-22A1
<i>tail up (tup)</i>	germ band shortening incomplete, head broad	2	—	—	54	
<i>thick head (thi)</i>	head broad	2	—	—	72	
<i>three rows (thr)</i>	denticles sparse, arranged in few rows	9	1	1	86	55A-F
<i>twist (twi)</i>	belts narrow, larva twisted, no ventral furrow in gastrulation	4	—	—	100	59B6-D5 ^c
<i>unpigmented (upi)</i>	unpigmented cuticle and mouth parts	4	1	—	93	
<i>u-shaped (ush)</i>	no shortening of germ band, lateral fusion of anterior and posterior hypoderm	2	—	—	0.1	21C8-E1
<i>wingless (wg)</i>	segment polarity mutant, mirror image duplication of denticle bands	6	—	1	30	
<i>zipper (zip)</i>	head and dorsal closure defective, small hole in ventral thorax	13	11	—	107.6	60E9-F1
<i>I(2)IA109</i>	dorsal anterior hole	1			60	
<i>I(2)IG76</i>	head broad	1	1		53	37F5-38A6,7
<i>I(2)JM45</i>	head skeleton glassy	1			86	55A-F
<i>I(2)IP85</i>	poorly differentiated cuticle and head	1			73	
<i>I(2)IIJ59</i>	head broad	1			49	
<i>I(2)II032</i>	head broad	1			27	

^a Map positions given in parentheses are approximate^b Cytology given in parentheses is based on translocation breakpoints only^c Spencer et al. 1982^d Wright et al. 1981^e Kornberg 1981^f Simpson 1983^g Ashburner et al. 1982

the head mutants. Of the head-mutants, 22 turned out to be allelic to one or the other of these seven loci. Complementation of all other mutants was shown by 14 mutants. Of these 7 proved to be either not fully penetrant or to have very small deviations from normal. These were assigned to the "subtle phenotype" class and not considered further. Two pairs of mutants were lethal in transheterozygotes, but failed to express an embryonic phenotype. They were regarded as not allelic.

Finally, after determination of the map position of most of the loci (see below), members of complementation groups mapping close to each other were crossed to ensure that we had not overlooked allelism between mutants with different phenotypes.

The complementation analysis outlined above led to the assignment of 259 of the 276 mutations into 48 complementation groups with 2–18 alleles. Four lines proved to be mutant in two separate loci. Seventeen mutants remained single. The "single" class, in addition to containing mutants at loci which have been hit only once by chance, is expected to contain all cases in which a phenotype is caused by an additive effect of two or more independent mutations, neither of which shows a significant phenotype by itself (synthetic phenotype). Recombination experiments (see below) showed that in at least 4 of these 17 mutants the phenotype did not map at a single point, but appeared to be synthetic. Two of these four cases had a head phenotype.

Our sample of 272 mutations with distinct embryonic

phenotypes defines a total of 61 loci on the second chromosome, 48 (79%) of which are represented by more than one allele. The arithmetic average is 4.5 alleles per locus. The distribution of allele frequencies is described in Fig. 2. It is note-worthy that the average allele frequency is significantly lower for loci with head defects as their predominant feature than for all other loci (see Discussion). Of the 14 "head" loci, 3 (21%) have more than two alleles, while the corresponding percentage for the 47 other loci is 67%. Of the latter, 83% have more than one and 17% more than seven alleles per locus. Mutants at three complementation groups are not embryonic lethal.

24 Genetic mapping

Members of each complementation group as well as the single mutants were mapped by recombination with visible markers as described in Materials and methods. Whenever possible, the back-cross was performed with an independent allele in order to complement other lethal functions which might have been induced simultaneously in the heavily mutagenized chromosome. In most cases the lethal function as well as the phenotype was mapped and the two sets of data were usually in good agreement. Mapping the lethal function in the 17 mutants with no known alleles showed that all but one had more than one lethal mutant per chromosome. In these cases, the mapping data are based solely on mapping of the mutant phenotype.

In order to assess allelism of some of our mutants with previously established loci, we inspected the lethal phenotypes of several dominant and recessive lethal mutants on the second chromosome as well as second-chromosomal

deficiencies (Table 1). Some of these showed an embryonic phenotype and, in cases in which allelism with members of our complementation groups was likely to be based on phenotype and map position, this was tested by complementation. Our mutant collection contains alleles at the previously known loci *Dopadecarboxylase* (Wright et al. 1976a), *engrailed* (Kornberg 1981), *Krüppel* (Gloor 1950; Wieschaus et al. 1984b), *Star* (Lindsley and Grell 1968), *wingless* (Babu 1977), *decapentaplegic* (Spencer et al. 1982), *crinkled* (Lindsley and Grell 1967; *l(2)br27* of Ashburner et al. 1982), and *snail* (*l(2) br28* of Ashburner et al. 1982). For the cytological location of some of the complementation groups, complementation tests were performed between chromosomes bearing a deficiency for the second chromosome and one or two members of complementation groups mapping within 10 map units of the deficiency. We tested 42 deficiencies covering a total of about 30% of the second chromosome and the approximate cytological location of 24 of the 61 loci was determined. Further, a number of translocations (Nüsslein-Volhard and Kluding, unpublished work), in which larger parts of the second chromosome segregated with the Y or third chromosome were tested as to whether the deficiency segregant uncovered the mutant phenotype. Since individuals hemizygous for large chromosomal deficiencies are usually embryonic lethal, showing phenotypes overlapping certain phenotypic classes of zygotic point mutants, only mutants with striking and unique phenotypes could be tested. The cytology of the chromosomal aberrations used is listed in Table 1 and illustrated in Fig. 3. The map positions of the 61 loci are indicated in Fig. 3 and Table 3.

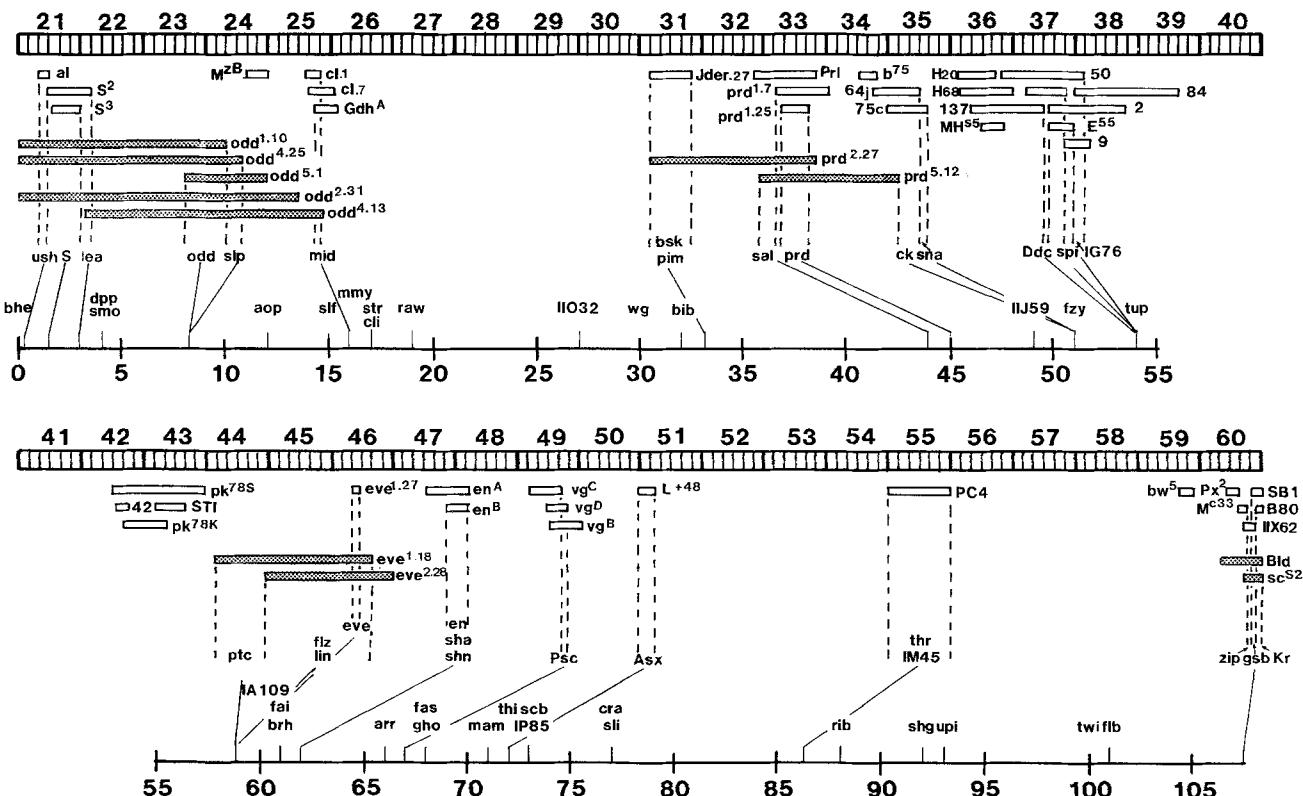


Fig. 3. A simplified map of the second chromosome indicating map position and cytological localization of the 61 loci. Deficiencies are represented by open bars, translocations by shaded bars. Cytologically undefined loci are shown directly above the genetic map. The more detailed cytological description of the chromosomal aberrations may be found in Tables 1 and 3.

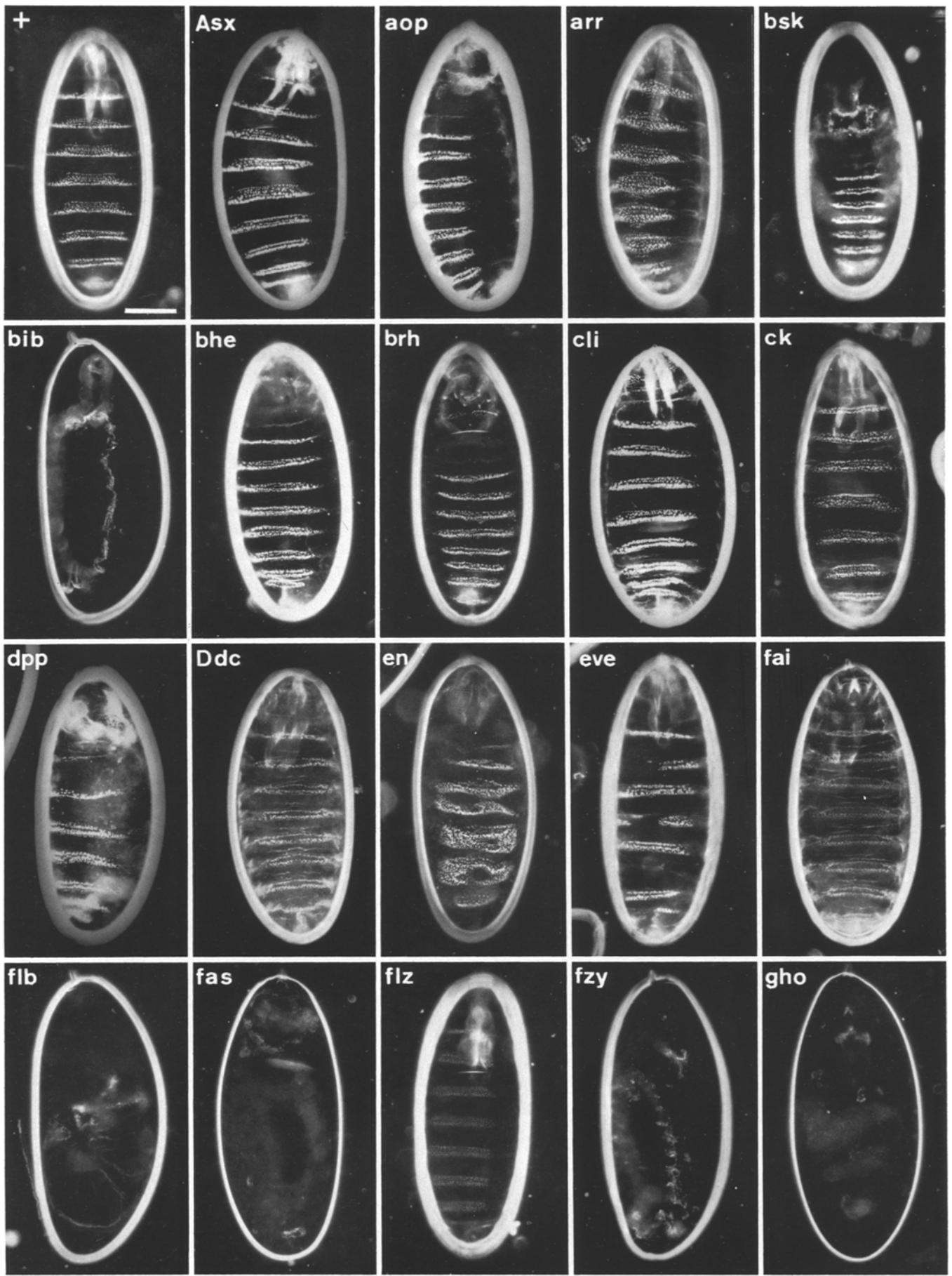
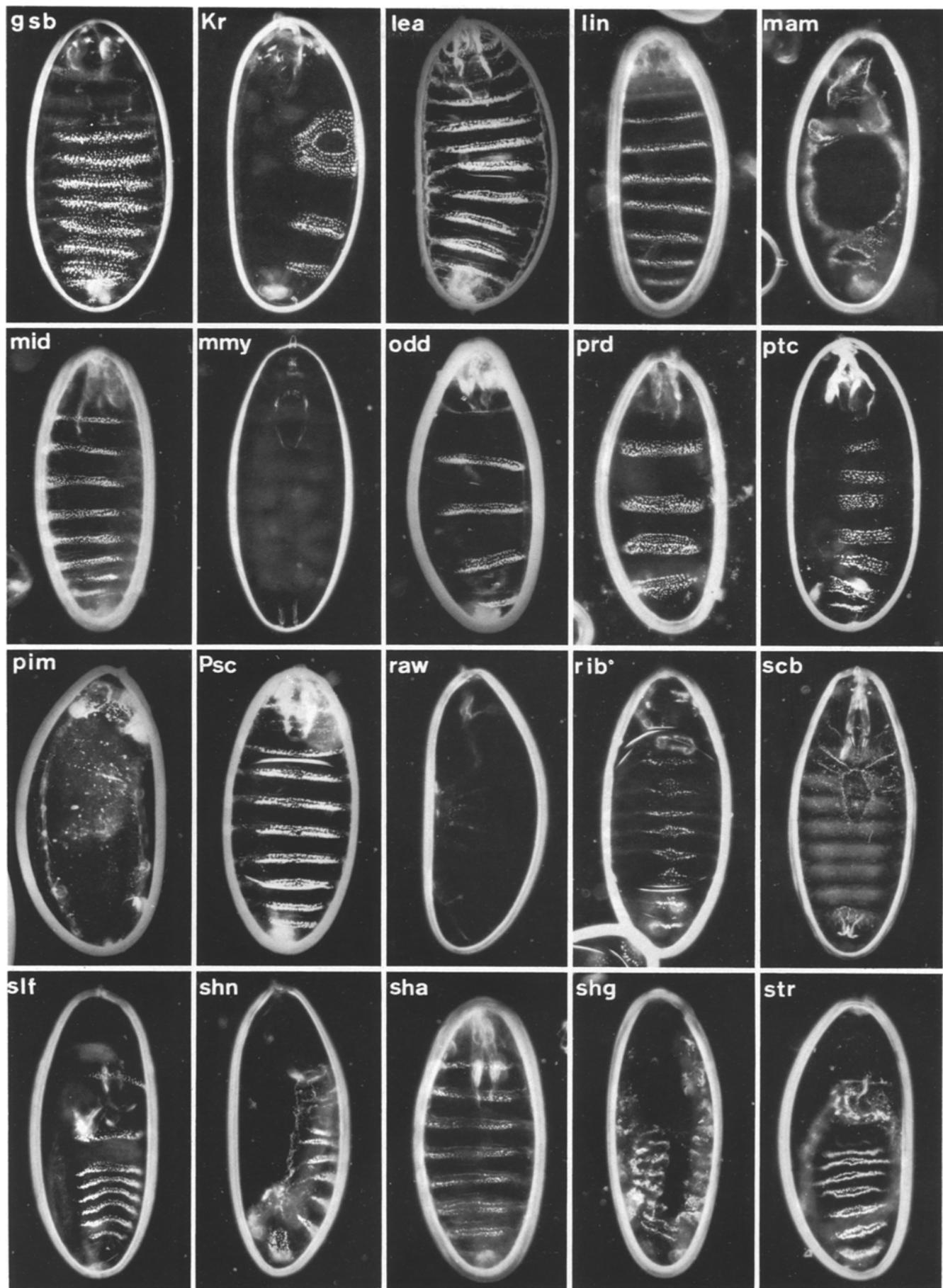
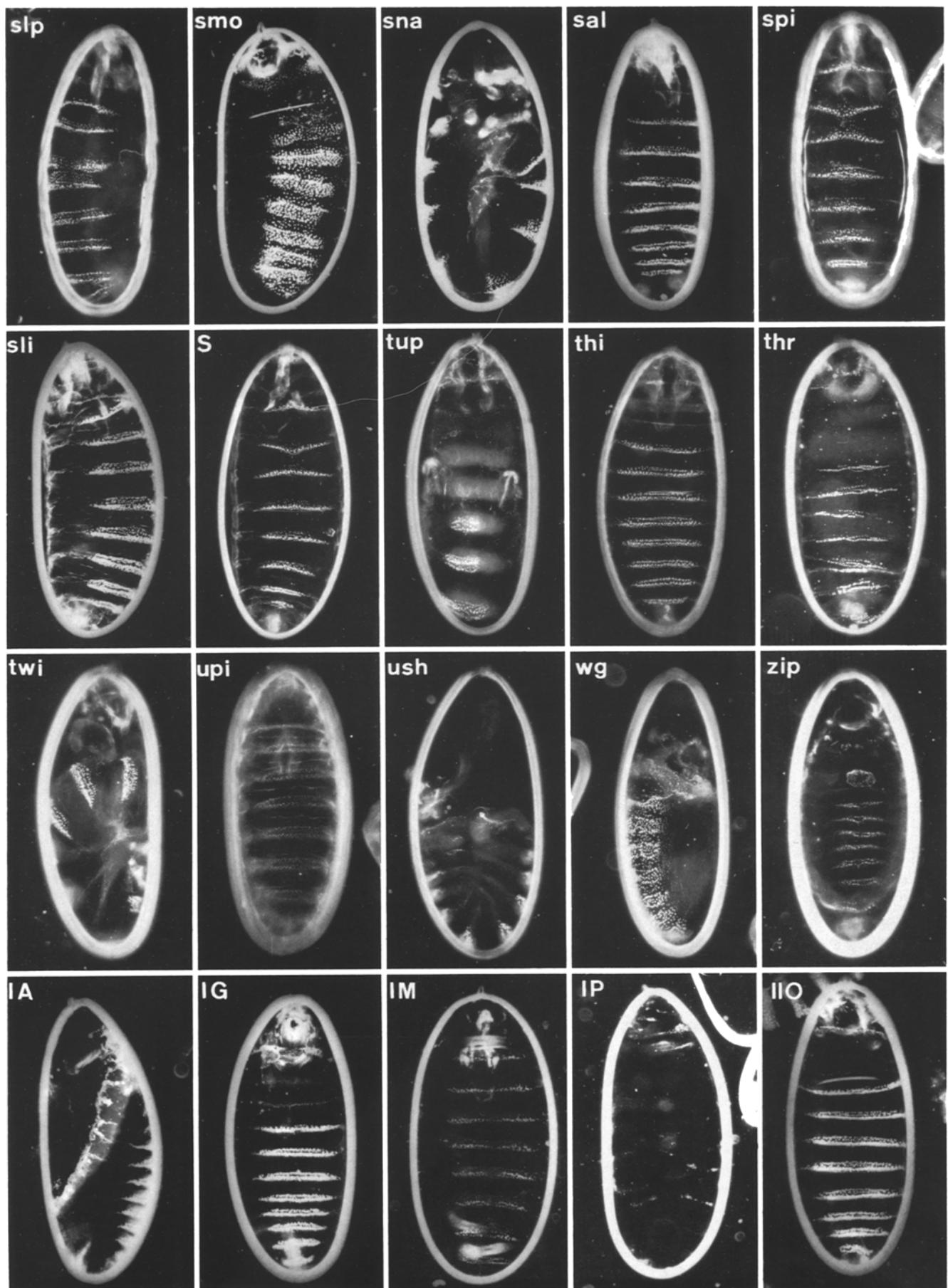


Fig. 4. Dark-field photographs of cuticle preparations of homozygous mutant embryos. A normal embryo, for comparison, is shown in the first picture. The *bar* represents 0.1 mm





25 Hypomorphic and temperature-sensitive alleles

As indicated in Table 3, in several complementation groups some alleles show a weaker phenotype than others. It appears likely that the strong phenotypes reflect the amorphic conditions of the respective genes, while the weaker phenotypes correspond to hypomorphic mutations. In several loci, temperature-sensitive (ts) alleles were found, which showed a weak or insignificant phenotype at low temperature and a strong phenotype at high temperature. Our screen has not been especially designed to isolate ts mutants, so our sample of 10 ts mutants per 272 mutants is probably under-represented.

In 13 instances it was possible to compare the true amorphic phenotype as represented by embryos homozygous for deficiencies of the respective loci with that of our alleles. In 9 of these cases, the strong alleles have the same phenotype as the respective deficiencies. For four loci all alleles have phenotypes weaker than the deficiency. In other cases, the homozygous deficiency phenotype was too poorly developed to discern differentiated structures (probably due to an additive effect of several lethal loci uncovered by the deficiency or a semi-dominant maternal effect associated with some of them (Garcia Bellido and Moscoso del Prado 1979). In these cases, transheterozygotes and strong alleles showed the same phenotype as the strong allele in the homozygous condition. Table 3 indicates the frequencies of the various types of alleles for all the loci.

Discussion

Phenotypic criteria

The work presented in the present paper is part of a programme aimed at identifying the genes involved in embryonic pattern formation in *D. melanogaster*. The differentiated embryo (larva) is a highly complex organism with many different cell types. In our screen for embryonic mutants we restricted ourselves to the analysis of the pattern of one particular tissue. We chose the larval epidermis, since the cuticle it produces is richly endowed with landmarks indicating position and polarity. The larval epidermis reveals perhaps the most striking aspect of embryonic pattern formation, segmentation, in greatest detail. The epidermis is derived from a large continuous region of the fate map and at hatching covers the entire larval body from anterior to posterior (Szabad et al. 1979; Lohs-Schardin et al. 1979). Thus it seemed likely that mutant effects on primarily soft parts of the embryo might also be detectable by a distortion or abnormal arrangement of the larval epidermis.

On the basis of a distinct phenotype visible in cuticular preparations, we have isolated 272 mutants mapping at 61 loci on the second chromosome. The mutant phenotypes cover a wide range of abnormalities. While the vast majority of the loci mutate to embryonic lethality, mutants at two of the loci, affecting the shape of denticles and hairs in the larva (*shavenoid* and *crinkled*) may survive to adulthood and in adults affect trichomes, and trichomes and bristles. Alleles at the *faint* locus are larval lethals. All other mutants are embryonic lethal and, in general, show a more severe deviation from normal than a mere morphological change in cuticle specializations. In many of the mutants internal organs are also strongly affected. From the analysis of the cuticular phenotype only, it often cannot be determined

whether the effect of the mutant gene on the epidermal pattern is a secondary effect of more general distortions. The mutant phenotypes are briefly described in Table 3 and illustrated in Fig. 4.

Many of the genes identified on the basis of their cuticular phenotype are not likely to be directly involved in embryonic pattern formation, but rather in final differentiation of embryonic primordia. On the other hand, we believe that the majority of genes affecting embryonic pattern formation should show a phenotype visible in cuticular preparations and thus (given the degree of saturation reached in our screen, see below) should be contained in our collection. The mutants we would not have detected in our screen are, on the one hand, those in which internal organs are missing or abnormally shaped, while the cuticular pattern is normal, and, on the other hand, those which do not develop to a stage advanced enough to show any signs of cuticular differentiation. The first type of mutants would have been scored "normal" or poorly differentiated, the second mistaken for unfertilized eggs and not identified as embryonic lethal.

A large number of the "normals" from our screen have been inspected for abnormalities in the central nervous system (CNS) of living embryos using Nomarski optics, and, apart from a few cases with an apparent failure in the condensation of the nervous system, no other phenotype could be detected (C.M. Bate, personal communication). A further sample of mutants listed in Table 2 under "subtle phenotypes" were scored likewise for CNS defects using fuchsin-stained whole mounts. One mutant was found that showed massive cell death in the CNS, similar to the phenotype of homozygous *scute* deficiencies (J. Campos-Ortega, personal communication). Apart from the CNS, however, no other internal organs have been carefully scored.

In order to assess the frequency of mutants of the second class – the ones stopping development before the appearance of cuticular structures – we scored a large number of lethal lines (corresponding to approximately 2000 lethal hits) for early phenotypes in living embryos and did not find any. An independent set of data is based on the phenotypes of embryos homozygous for chromosomal deficiencies. The set of deficiencies on the second chromosome covers about 30% of the bands and all but one (*Df(2)vg^B*) show a phenotype which we would have detected in our screen or scored as normal or poorly differentiated. In cuticular preparations, *vg^B* embryos are indistinguishable from unfertilized embryos, whereas all other deficiencies tested, even very large ones, show at least signs of differentiation and many develop a normal cuticular pattern (Table 1).

The degree of saturation for embryonic visible phenotypes

Several lines of argument indicate that we have identified most of the loci on the second chromosome that mutate to an embryonic visible phenotype.

Rate of discovery of new loci. A simple calculation is based on the rate of isolation of mutants at new loci per chromosomes scored. Figure 5 shows that after scoring the first 25% of all lines, at least one mutant in more than 50% of the finally identified loci had been found, while in the last 25% of the lines only three new loci (5%) were found. The frequency of total mutants per lines scored was con-

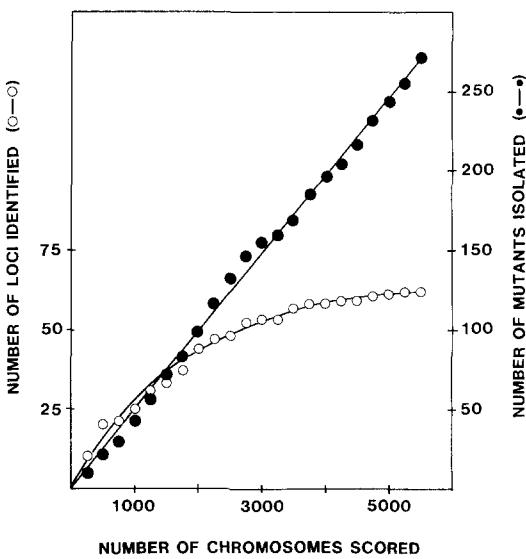


Fig. 5. The frequency of discovery of new loci compared to the total number of mutants isolated during the screen

stant throughout the screen. Thus, at the end of the screen alleles of previously isolated mutants were predominantly found. In a subsequent screen for third-chromosomal lethals (Jürgens et al. 1984), which also allowed the detection of second-chromosomal mutations with striking phenotypes (predominantly segmentation mutations), we found only alleles of previously identified loci.

Allele frequencies. Of the 61 loci, 79% are represented by more than one allele, the average being 4.5 alleles per locus. We assume that the majority of the mutations in loci represented by more than one allele are amorphic or hypomorphic mutations. Among the mutations in the 13 loci represented by only one allele, on the other hand, several might be antimorphic or neomorphic mutations in genes where amorphic mutations have no significant phenotype or are not embryonic lethal. Furthermore, the single allele class may also include other rare events like double mutations, neither of which produces a significant phenotype by itself. Our recombination experiments allowed the detection of synthetic phenotypes only if the individual mutants mapped rather far apart, and of the 17 initial single mutants 4 were found to be synthetic. Another possibility is that some single mutants are EMS-induced deficiencies and the phenotypes are caused by a cumulative effect of several genes of the poorly differentiated class. Therefore, estimations of the zero class based on the frequency of loci represented by one allele must be viewed with caution, since the one allele group is probably artificially enlarged.

The distribution of allele frequencies per locus (Fig. 2) is not random. Some loci appear to be mutable in much higher frequencies than others, the highest allele frequency exceeding the average by a factor of 4. On the other hand, allele frequencies might be severely biased in certain cases by the detectability of the phenotype in weak alleles. For instance, if an amorphic mutation in a certain locus leads to only a slight deviation from normal, a hypomorphic mutation in the same gene might not show a significant phenotype or might not even be embryonic lethal and thus go undetected. Similarly, loci with very strong extreme phenotypes may be detectable in comparatively weak alleles. The

five 'hot spots' (*ptc*, *zip*, *flb*, *shn* and *shg*) all show very strong extreme phenotypes and are all represented by a number of weak alleles. The low allele frequency of most of the loci which show head defects as the major phenotypic feature might be explained by a similar argument. Head involution is a particularly sensitive process in embryogenesis in that slight disturbances lead to gross distortions, while even slighter ones may allow normal development.

While our screening criteria might have discriminated against weak alleles in several cases, it is possible that in other cases we have discriminated against strong alleles, if these show haplo-insufficient lethality or semi-lethality. It is striking that of the four loci for which we could show by comparison with the deficiency phenotype that our screen produced only weak alleles (*eve*, *Kr*, *prd* and *IG76*), three show semi-dominant lethality in hemizygous condition. *eve* is probably haplo-insufficient semi-lethal (Nüsslein-Volhard, unpublished work), *Kr* has a semi-dominant larval and adult phenotype (Gloor 1950; Wieschaus et al. 1984b) and deficiencies uncovering *IG76* have a semi-dominant maternal effect. These data indicate that in our screen we have discriminated in particular cases against certain types of alleles (depending on the locus; amorphic or hypomorphic). Thus the average allele frequency obtained is probably lower than the true hit frequency per locus.

In addition to the different level of detectability of different mutant phenotypes and probability considerations, the allele frequencies are likely to reflect the mutability of some of the genes with EMS. For 5 of the 13 loci represented by one allele in our screen, pains have been taken by others and by us to isolate more alleles. While the loci *sna*, *slp* and *Asx* (Ashburner et al. 1982; Simpson, personal communication; Jürgens et al. 1984; Jürgens, personal communication) seem to be mutable at a normal rate, EMS induced *dpp* alleles are very rare (Spencer et al. 1982), probably reflecting the haplo-insufficient lethality of the locus. The single *gsb* allele isolated in our screen turned out to be a small deletion (*Df(2R)IX62*). In an EMS screen for *gsb* and *Kr* alleles, Preiss and Jäckle (personal communication) tested close to 20000 lethal hits and recovered 10 *Kr* alleles, one further *gsb* deletion but no single *gsb* point mutant. On the other hand, since all other significant deficiency phenotypes are accounted for by point mutants isolated in our screen, and this holds also for deficiencies on the third and first chromosome (Jürgens et al. 1984; Wieschaus et al. 1984a), non-mutability with EMS seems to be the exception rather than the rule.

Comparison with deficiency phenotypes. For the second chromosome, deficiencies covering about 30% of the bands have been tested for their homozygous lethal phenotype (Table 1). All significant embryonic visible phenotypes revealed in deficiency homozygous embryos have been accounted for by point mutants uncovered by the deficiencies, with the exception of *gsb* mentioned above. Of the 61 loci, 17 could be cytologically mapped using the available deficiencies which had been isolated by criteria other than an embryonic visible phenotype. This number (29%) corresponds well with the 30% of the bands uncovered by these deficiencies.

Calculation of the total number of lethal loci on the second chromosome. Given the saturation of the second chromosome for one particular phenotype, the total number of

lethal genes on the second chromosome may be calculated. The frequency of chromosomes having received at least one lethal hit in our experiment was 73% (4217/5764). Assuming a Poisson distribution of lethal hits, we can calculate the total number of lethal hits induced using the formula $P(0) = e^{-\mu}$ whereby $P(0)$ is the fraction of chromosomes without a lethal hit (0.27) and μ the average number of hits per chromosome. The calculation yields a total number of about 7600 lethal hits, 272 of which (3.6%) yielded an embryonic visible phenotype defining 61 loci. Assuming equal mutability of all lethal loci, these 61 loci should correspond to 3.6% of all lethal loci on the second chromosome. This calculation yields a total number of about 1700 lethal loci on the second chromosome, which is in fair agreement with estimates based on the number of bands (Lefevre 1974; Garcia-Bellido and Ripoll 1978).

Conclusions

In this paper we have described the isolation of mutants at 61 loci on the second chromosome of *D. melanogaster*. Some of the loci were known previously but most of them are new. All of them affect the formation of a normal cuticular pattern during embryogenesis. Although we cannot claim to have reached saturation for such loci in our screen, our calculations presented above indicate that our collection represents the great majority of such loci especially of those which mutate to a striking and unique embryonic visible phenotype. The number of genes which mutate to an embryonic visible phenotype is surprisingly small. For the second chromosome, these genes represent about 3% of all loci (approximately 2000) and similar numbers are obtained for the third and first chromosome. **This means that the expression of the vast majority of the genes during embryogenesis is dispensable for the formation of a morphologically normal larva.**

It is not the purpose of the present paper to describe the mutant phenotypes. Several of the phenotypes have been studied in some detail: those of the loci involved in segmentation (Nüsslein-Volhard and Wieschaus 1980; Nüsslein-Volhard et al. 1982), *Krüppel* (Gloor 1950; Wieschaus et al. 1984b) *engrailed* (Kornberg 1981), those of the loci of early neurogenesis *mam* and *bib* (Lehmann et al. 1983), and the phenotype common to the loci *twist* and *snail* (Frohnhofer 1982; Simpson 1983) which, of all loci, shows the earliest visible deviation from normal, the lack of the ventral furrow during gastrulation. A superficial inspection of the pictures of mutant embryos in Fig. 4 shows that the degree to which the mutant pattern deviates from the normal pattern is great in some and barely detectable in others. From this picture it also becomes apparent that the genes described here are of very different importance for embryonic pattern formation. A comparison of the mutant phenotypes as shown in cuticular preparations further indicates that the majority of the genes have their own distinct phenotype. We estimate that the 61 loci of the second chromosome represent at least 41 distinguishable phenotypes and by a more detailed histological and developmental analysis of the mutant phenotypes this number may increase substantially. On the other hand, several of the mutant phenotypes are shared by loci mapping on the third or first chromosome (Jürgens et al. 1984; Wieschaus et al. 1984a). No clustering of genes with similar phenotypes is apparent; instead, the loci with embryonic visible pheno-

types are spread more or less randomly throughout the genome.

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Notes added in proof

- 1) The locus *unpigmented* appears to be identical with the *Punch* locus (Lindsley and Grell 1968), based on complementation tests with *Pu*² and a dominant eye-colour phenotype of *upi* alleles
- 2) One representative allele of each locus can be obtained from the following address: Mid-America Drosophila Stock Center, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, USA