Cytogenetic Definition and Morphogenetic Analysis of Delta, a Gene Affecting Neurogenesis in Drosophila melanogaster

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ABSTRACT

We have conducted a genetic analysis of a small interval of the third chromosome known to include Delta (Dl), a locus that affects the segregation of the ectoderm into neural and epidermal lineages during embryogenesis and the morphogenesis of some ectodermally derived structures, in Drosophila melanogaster. This analysis has led to the definition of seven independent complementation groups, one of which is Delta, within the interval extending from 91F6-13 to 92A2. Among the extant mutations in these seven loci, only mutations in Dl lead to the so-called neurogenic phenotype: hypertrophy of the nervous system and reduction of the epidermis. Combined cytogenetic and genetic analyses allow us to define absolute proximal (91F5-92A1) and distal (92A2) cytogenetic limits for the Dl locus. We have isolated hypomorphic and amorphic alleles of Dl and find that, for any given allele, there is an inverse correlation between neural hypertrophy and epidermal reduction in embryos and a direct correlation between the severity of embryonic phenotypes in mutant homozygotes and hemizygotes and the imaginal phenotype in heterozygous adults.

NEUROGENESIS in Drosophila is first apparent shortly after gastrulation when a subset of the cells within the ectoderm segregate toward the interior of the embryo. These cell movements occur concomitant with pronounced changes in cell volume and shape within an area of the embryo defined as the neurogenic region (POULSON 1950). The cells that have segregated inward develop into neuroblasts, from which neural cells within the nervous system are derived. The ectodermal cells remaining on the exterior surface of the embryo develop into dermomyoblasts which ultimately give rise to the epidermal cells that secrete larval and adult integuments. Neuroblasts are recruited from blastoderm cells without intervening mitoses indicating that the commitment of a subset of cells to the neural lineage probably occurs before there is morphological evidence of neurogenesis (CAMPOS-ORTEGA and HARTENSTEIN 1985).

POULSON (1937) observed that embryos hemizygous for a deficiency removing the Notch (N) locus exhibited a grossly hypertrophied central nervous system, severe reduction in the amount of epidermal tissue formed and embryonic lethality. On the basis of its affect on neurogenesis, POULSON designated N as a neurogenic gene. The result was interpreted as indicating that the loss of function of the N product led to the developmental misrouting of cells within the ectoderm such that the cells that would normally enter the epidermal lineage instead entered the neural lineage. WRIGHT (1970) suggested that cells within the neurogenic region are initially equipotent, being committed to the neural lineage, and that the N product acts to restrict a significant fraction of the ectodermal cells from entering the neural lineage. These developmentally restricted cells then enter the epidermal lineage. Thus, loss-of-function mutations in N result in an increase in the fraction of ectodermal cells within the neurogenic region that enter the neural lineage via the elimination of an otherwise functional developmental restriction. Over the past 50 yr, work by a number of investigators has led to the definition of a set of loci, the zygotic neurogenic genes, each of which plays an essential role in the regulation of the commitment to neural and epidermal lineages within the embryonic ectoderm. A set of six zygotic neurogenic genes has been defined: Notch, Delta (Dl), Enhancer of split [E(spl)], big brain (bib), mastermind (mam), and neutralized (neu) (POULSON 1937; LEHMANN et al. 1983; NÜSSLEIN-VOLHARD, WIESCHAUSS and KLUDIG 1984; JÜRGENS et al. 1984; WIESCHAUSS, NÜSSLEIN-VOLHARD and JÜRGENS 1984).

We have conducted a genetic analysis of a chromosomal interval extending from 91F5 to 92A2 on the right arm of the third chromosome that includes the zygotic neurogenic locus Dl [chromosome 3R, map position 66.2 (LINDSLEY and GRELL 1968); 92A1 (A. K. ALTON, C. C. KOPCZYNSKI, K. FECHTEL and M. A. T. MUSKAVITCH, unpublished data)]. Loss-of-function mutations in Dl lead to recessive embryonic lethality and the characteristic embryonic neurogenic
phenotype: nervous system hypertrophy and reduction in the epidermis. Adult animals heterozygous for a Dl loss-of-function mutation and a wild-type allele exhibit a dominant phenotype consisting of disrupted wing venation. A considerable number of Dl mutations, resulting from spontaneous mutation or induced following X-ray or chemical mutagenesis, have been isolated on the basis of this dominant wing phenotype (Lindsley and Grell 1968; Lehmann et al. 1983; Vassil and Campos-Ortega 1987; A. L. Terry and M. A. T. Muskavitch, unpublished data). Although a number of these alleles are available to us, we decided that it was important to isolate mutations on the basis of an alternative criterion, recessive lethality, since it is conceivable that mutations could arise within the locus that would be lethal but not yield a dominant wing phenotype in heterozygotes. The screen we undertook also permitted identification of a number of lethal complementation groups flanking Dl.

We describe the results of a second generation (F2) lethal screen employing ethylmethane sulfonate (EMS) mutagenesis in which we recovered 29 independent recessive lethal and semilethal mutations uncovered by a portion of the deficiency Df(3R)ChaM9, which includes the region 91F5-92A2. The genetic analysis of these mutations permits an initial description of the genetic complexity of Dl and its flanking regions and facilitates the molecular analysis of the Dl locus in which we are currently engaged. Furthermore, this analysis adds to the number of small chromosomal intervals in Drosophila for which the distribution and arrangement of lethal and semilethal mutations has been investigated (Judd, Shen and Kaufman 1972; Woodruff and Ashburner 1979; Nicklas and Cline 1983; Crosby and Meye-rowitz 1986; Vassil and Campos-Ortega 1987).

MATERIALS AND METHODS

Drosophila stocks: The deficiencies used in this study are listed in Table 1. Unless otherwise noted below, markers are described in Lindsley and Grell (1968). The Oregon-R stock used was obtained from D. S. Hogness, Stanford University and the Canton-S strain from W. J. Welshons, Iowa State University. The dominant mutation Tubby (Tb) leads to the formation of short, squat larvae, pupae and adults (Craymer 1980). The third chromosome balancer TM6B, Hu and Tb ca (TM6B) was created and described by Craymer (1984). The chromosome TM6B, Hu Df(3R)88 and Tb ca (TM6B,Dl) was generated in our laboratory in a screen for haploabnormal Dl alleles using EMS as a mutagen (M. A. T. Muskavitch, unpublished data). Tp(3)MKRS, M(3)834 kar2 mut 28 (MKRS) is an effective balancer for a portion of chromosome arm 3R comprising the interval from division 87 through division 93 (Hilliker et al. 1980). The spontaneous apparent point mutation D1 is described in Lindsley and Grell (1965). Mutations were induced on an isogenic third chromosome marked with mutations in spineless, ebony, and rough (ss e ro) which was constructed in our laboratory. The stock ss e ro ca was derived from this isogenic stock by recombination to add the marker claret.

Cultured conditions: Drosophila cultures were raised at 25° (unless otherwise noted) on standard cornmeal-molasses-yeast-agar medium seeded with live baker's yeast.

Mutagenesis: ss e ro males were aged for 48 hr at ambient temperature (22–25°) and then fed a solution of 0.0125 M EMS (Sigma) in 2% (w/v) sucrose, following a modification of the method of Lewis and Bacher (1968). After 24 hr of feeding at ambient temperature, males were transferred without etherization into fresh culture vials containing standard medium and mated en masse to virgin Df(3R)ChaM9, kar2 ro red ChaM9/MKRS females (approximately 15 males and 40 virgins per vial) for 24 hr. Males and females were then separated, and inseminated females were allowed to lay eggs on fresh media in milk bottles for three intervals of 2 days. First generation (F1) males of the appropriate genotype were collected 0–24 hr after eclosion and aged for several days before mating. The scheme used to recover lethals in the 91F5-92A2 region is presented in Figure 1. Individual ss e ro Df(3R)ChaM9, kar2 ro red ChaM9 F1 males were mated in vials to six to eight virgin females at 25° (30% of the crosses) or 29° (70% of the crosses). After 7 days, parents were cleared from the vials. On day 17, individual females were scored by looking through the sides of the culture vials. The absence of empty Tubby+ pupal cases was taken as an indication of the presence of a lethal mutation within the region spanned by Df(3R)ChaM9 and unaffected by Df(3R)ChaM9. Phenotypes of Tubby+ individuals from crosses that yielded empty Tubby+ pupal cases were not assessed; thus, Dl alleles that initially exhibited a significant level of viability over Df(3R)ChaM9 were not recovered. Mutant lines were established from single lethal-bearing third chromosomes for each allele and maintained in stocks heterozygous for TM6B. Each lethal mutation, balanced in stock over TM6B, was subsequently retested for lethality over Df(3R)ChaM9. Mutations were

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<td>Df(3R)ChaM9(1)</td>
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<td>89E2-3; 91D1; 92A5</td>
<td>E. B. Lewis</td>
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Unpublished data.

Deficiency segregate of Tp(3)kod(110) (Lindsley and Grell 1968); isolated from the transposition by recombination in our laboratory.

Bridges and Brehm (1944).

Lehmann et al. (1983).
also tested for lethality when heterozygous with \( Df^{6} \). Mutations were designated \( BE \) (for Bloomington, EMS-induced) and assigned an arbitrary allele number.

**Deficiency mapping and assignment of mutations to complementation groups:** All the lesions we recovered as lethal or semilethal mutations over \( Df(3R)Cha^{-} \) were divided into two groups based on complementation behavior in combination with \( Df(3R)Cha^{M} \), which subdivides the interval spanned by \( Df(3R)Cha^{M} \) (Figure 2). One group of mutations was lethal over both \( Df(3R)Cha^{M} \) and \( Df(3R)Cha^{M} \), while the second group was lethal over \( Df(3R)Cha^{M} \) and viable over \( Df(3R)Cha^{M} \). Mutations mapping to the same subinterval were tested inter se for complementation in all possible pairwise combinations. Test crosses were done in only one polarity, chosen on a random basis, for each allele pair. A minimum of 100 progeny were scored for each cross. The absence of spineless-" ebony-", "Tubby", rough-" pupae and/or adult flies was used as the criterion for failure of complementation. Lethality was defined as viability of heterozygotes at a level less than 5% of expectation. Semilethality was defined as viability of heterozygotes at a level between 5% and 15% of expectation. Pupal lethality was defined as viability to pharate adulthood at a level greater than the semilethal criterion and eclosion at a level less than the lethal criterion.

Seven complementation groups were defined in this manner, one of which contained mutations that fail to complement additional \( DI \) alleles (Figure 2). The six \( DI \)-proximal lethal complementation groups, which map to the cytological region 91F, were assigned alphabetical designations in accord with current convention (Lindsley and Zimm 1986). The order of assignment of the designations (\( a \) through \( f \)) followed the ascending order of allele numbers for prototypic alleles of individual complementation groups. Representatives from each of the six \( DI \)-proximal complementation groups were tested for complementation in pairwise combination with each of the \( DI \) noncomplementing mutations.

**Meiotic mapping:** Each of the mutations that failed to complement \( DI \) alleles was mapped meiotically according to the scheme outlined in Figure 3. Males heterozygous for a \( DI \) mutation and the balancer \( TM6B \) were outcrossed to Oregon-R virgins. Virgins heterozygous for the desired \( DI \) allele and an Oregon-R third chromosome were collected and mated to \( ss \) to ca males. Single males bearing recombinant chromosomes representing crossovers within the \( s-s \) or the \( e-e \) intervals or apparent nonrecombinant chromosomes were mated in vials to six to eight \( Pr/TM6B, DI \) virgins, and crosses were scored for complementation between the recombinant chromosome and the \( DI \) mutation on the balancer chromosome. In the case of \( BE38 \), the scheme used for meiotic mapping differed slightly due to the presence of a spontaneous \( ca \) mutation on the chromosome carrying this allele (see legend to Figure 3).

**Cytology:** Males from balanced stocks (\( Df^{6}/TM6B \)) were outcrossed to either Oregon-R or \( ge^{l} \) \( w^{e} \) virgins, and the resulting larvae were raised at 18°. Larvae wildtype for body morphology ("Tubby") were selected for analysis since this class was expected to possess chromosomes carrying the mutation of interest. Temporary salivary gland squashes...
Generation of embryos for morphological analyses:
Either of two types of crosses were employed to generate embryos for analysis. For the first type of cross, males and females from a stock heterozygous for the Dl allele of interest and the balancer TM6B were crossed inter se to yield embryos homozygous for the allele of interest. For the second type of cross, males of the genotype Dp/TM6B were crossed to Oregon-R virgin females, and males of the genotype Df(3R)De3/TM6B were crossed to Canton-S virgin females. The resulting Dl/+ males, from the cross involving Oregon-R, were crossed to Df(3R)De3+/+, from the cross involving Canton-S, to yield embryos hemizygous for the Dl allele of interest.

Direct immunofluorescence of embryo whole mounts:
The procedure is a variation, developed by N. H. Patel and C. S. Goodman (Stanford University), of the method described in Karr and Alberts (1986). We include additional minor modifications. All steps are performed at ambient temperature, unless otherwise noted. Crosses of appropriate genotype were conditioned in bottles on heavily yeasted fly food for two or three days at 25°C. Fertilized eggs were collected for an eight hour interval at 18°C on grape juice agar plates (Elgin and Miller, 1978) seeded with dry yeast; adults were removed and the plates were placed at 18°C for 12-16 hr. Developing eggs (50-300) were transferred to 12 mm x 75 mm silanized (Sigma Cote, Sigma Chemical Co.) glass culture tubes, dechorionated in 50% (w/v) household bleach in water for 5 min and rinsed extensively with 0.02% (v/v) Triton X-100 in deionized water. Following removal of the water by aspiration, the embryos were fixed by suspension in 0.5 (ml) 4% (w/v) paraformaldehyde to which 1.5 ml of heptane were added. This suspension was vortexed intermittently for 10-20 min. The lower phase was removed by aspiration and 0.5 ml of 100% methanol was added for 1-2 min to remove vitelline membranes from the embryos. Devitellinized embryos sink to the bottom of the methanol phase. Heptane and methanol were removed by aspiration, and the embryos were rinsed twice in 1.5 ml 100% methanol and placed at 18°C for 12-16 hr.

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Slides were sealed with clear nail polish and stored in the dark at either 4°C or ambient temperature. Preparations were viewed using a Zeiss Universal microscope equipped for epifluorescence with a 100 W mercury source and a Zeiss 48 77 09 (transmittance 450-490 nm) filter set. Stained embryos were photographed using ISO 400 FujiChrome or Fujicolor film at exposures of 30-120 sec.

Cuticular preparations:
Embryonic cuticles were prepared for analysis using the method described by Hoffmann and Goodman (1987), except that vitelline membranes were prepared by dissection of salivary glands from third instar larvae in TB1 (Bonner et al. 1984) saline, followed by fixation and staining in 2% (w/v) orcein in 1:1 lactic acid:glacial acetic acid. Cytological preparations were compared to the maps of Leffere (1976).
Table 2: Interallelic complementation among Delta alleles

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Polarties of interscrossoes among newly induced DI alleles (BE30–BE39) were chosen at random. Complementation tests with previously induced DI alleles (bxdM10, M2, M3, CE14) involved balanced males carrying newly induced DI alleles and balanced virgins carrying previously induced DI alleles. Numerical criteria for different complementation behaviors are described in MATERIALS AND METHODS.

RESULTS AND DISCUSSION

Mutations isolated: The goal of the screen we undertook was two-fold. First, we wanted to isolate a set of DI alleles sufficiently altered in DI function to be identified on the basis of recessive lethality, independent of any associated dominant Delta wing phenotype in heterozygotes. In this manner, we hoped to recover DI mutations which would have been undetected in our previous F1 screens in which mutants were selected on the basis of the dominant wing phenotype (A. L. TERRY and M. A. T. MUSKA-VITCH, unpublished data). Second, we wanted to isolate mutations in loci within the chromosomal region flanking the DI locus in order to determine whether any functionally related genes were located in the vicinity of DI, as well as to assist us in our ongoing molecular analysis of the region.

From a screen of 12,769 EMS-mutagenized chromosomes, outlined in Figure 1, we have isolated ten recessive lethal or semilethal DI mutations (Figure 2). Each of these ten mutations (BE30–BE39) was determined to be allelic to DI by its failure to complement the apparent point mutation DI, and some or all members of a set of known DI deficiencies (Df(3R)ChaM1, Df(3R)DfM2, and Df(3R)bxdM10). Furthermore, most of these new DI alleles fail to complement one another (Table 2). We have also isolated 19 independent recessive lethal or semilethal mutations that fall into six other independent complementation groups. We have mapped these complementation groups to the chromosomal interval immediately proximal to DI using deficiencies with breakpoints that subdivide this interval (discussed below and Figure 2).

Deficiency mapping: All 29 independent recessive lethal or semi-lethal mutations recovered in this screen were tested for their ability to complement a series of overlapping deficiencies (Table 1, Figure 2) with breakpoints that subdivide a portion the interval uncovered by Df(3R)ChaM1, the deficiency over which these lesions were originally identified as lethal mutations. Based on the results of deficiency mapping, the 29 mutations recovered could be divided into two groups (Figure 2). The first group comprises 10 mutations that failed to complement known DI alleles, but did complement Df(3R)ChaM1 which possesses a distal breakpoint within the 91F5-13 interval, immediately proximal to the DI locus. These mutations represent apparent DI alleles and map distal to the distal breakpoint of Df(3R)ChaM1. The second group comprises the remaining 19 recessive lethal or semi-lethal mutations, all of which were shown to complement the point mutation DI, but failed to complement Df(3R)ChaM1. This latter group must therefore map to the left of DI and proximal to the distal breakpoint of Df(3R)ChaM1. Thus, the distal breakpoint of Df(3R)ChaM1, 91F5-92A1, defines an absolute proximal genetic, and therefore physical, limit for DI.

We were able to further order the lethal complementation groups in the interval proximal to DI by complementation analysis employing a set of three
deficiencies \((\text{Df}(3R)\text{D}^{bx}12, \text{Df}(3R)\text{D}^{H128})\) which subdivides the distal portion of \(\text{Df}(3R)\text{Cha}^{M1}\) (Figure 2). In this manner, we have determined that among the six lethal complementation groups proximal to \(\text{DI}\) either \(l(3)\text{91Fd}\) or \(l(3)\text{91Ff}\) maps immediately proximal to \(\text{DI}\). The occurrence of molecular lesions corresponding to mutations affecting these complementation groups may prove useful in the subsequent molecular definition of a proximal physical limit for \(\text{DI}\).

A distal limit for \(\text{DI}\) can be defined by analyzing the components of the transposition \(\text{Tp}(3)\text{bx}^{d10}\). The deficiency segregant \(\text{Df}(3R)\text{bx}^{d10}\) eliminates \(\text{DI}\) function (Lindsley and Grell 1968), but the intact transposition is wild type for \(\text{DI}\) function. These conclusions are supported by our observations that the transposition chromosome is viable in heterozygous combination with \(\text{DI}^{91}, \text{DI}^{E30}\) and \(\text{Df}(3R)\text{D}^{M2}\) while the deficiency segregant is lethal when heterozygous with each of these alleles. We therefore infer that the duplication segregant of the transposition carries the intact wild-type \(\text{DI}\) function. In order to further localize the duplicated copy of the \(\text{DI}\) locus on the transposition chromosome, we replaced the majority of the transposition chromosome (approximately 97% of the cytological map) with segments of a wild-type third chromosome by directed meiotic recombination (data not shown). The recombinant chromosome \(\text{Ds}(3;3)\text{bx}^{d10} \times \text{taxi, map position 91, Lindsley and Grell 1968}\), which should carry two intact copies of \(\text{DI}\), was then shown to complement the haploabnormal wing phenotype associated with heterozygosity for \(\text{DI}^{91}\). In this manner, we have localized the intact copy of \(\text{DI}\) to an interval between \(\text{ss}\) and \(\text{stripe}\) (\(\text{sr}\), Lindsley and Grell 1968) on the transposition chromosome. This inference, which should be valid assuming no cytologically cryptic copies of the \(\text{DI}\) locus reside on the transposition chromosome within the \(\text{ss} -\text{sr}\) interval, is in agreement with a previous report by E. B. Lewis (Lindsley and Grell 1968) concerning the duplication segregant of this transposition. Thus, the distal breakpoint of \(\text{Df}(3R)\text{bx}^{d10}, \text{92A2}\), defines an absolute distal genetic and physical limit for \(\text{DI}\).

**Complementation analysis:** All 19 independent recessive lethal or semi-lethal mutations that mapped proximal to the \(\text{Df}(3R)\text{Cha}^{M1}\) distal breakpoint, and therefore proximal to \(\text{DI}\), were mated inter se in all possible pairwise combinations. The data indicated that the 19 mutations could be grouped into six independent complementation groups (Figure 2). A single representative allele was chosen from each of the six complementation groups and tested inter se with each of the ten recessive lethal or semi-lethal \(\text{DI}\) mutations we had isolated for complementation behavior. The representative chosen from each of these six complementation groups complements all ten \(\text{DI}\) alleles tested. This genetic behavior suggests that the 29 mutations we have isolated define seven independent complementation groups and that none of the alleles we have isolated represent multiple-hit events. We also observe no striking effects on imaginal phenotype in transheterozygotes bearing any one of these \(\text{DI}\)-proximal lethal mutations in combination with any of a number of \(\text{DI}\) alleles tested other than the appropriate Delta™ phenotype. It would appear that none of the six loci immediately proximal to \(\text{DI}\) interact with this neurogenic gene, as assessed by analysis of the alleles we have in hand. The mutations we have isolated therefore fall into seven complementation groups: one of these is \(\text{DI}\) and the other six map proximal to \(\text{DI}\).

We note that our screen of over 12,000 mutagenized chromosomes has led to the definition of six discrete lethal complementation groups proximal to \(\text{DI}\). Representative members of each complementation group fail to be complemented by \(\text{Df}(3R)\text{D}^{M3}\), a deficiency that removes the \(\text{Dl}\) locus and extends proximally into the distal portion of polytene band 91F (Table 1). Vassim and Campos-Ortega (1987) screened a similar number of mutagenized chromosomes for lethal mutations in the interval removed by this same deficiency and defined four complementation groups proximal to \(\text{DI}\) within \(\text{Df}(3R)\text{D}^{M3}\). The contention that Vassim and Campos-Ortega achieved lethal saturation of this \(\text{DI}\)-proximal interval cannot be supported by our data.

Although our screen was also designed to detect lethal or semi-lethal mutations that map within \(\text{Df}(3R)\text{Cha}^{M9}\) but distal to \(\text{DI}\), no such mutations were detected. A similar analysis by Vassim and Campos-Ortega (1987) led the authors to suggest that the region immediately distal to \(\text{DI} (92A1-2 \text{ to } 92A8-10)\) contains no loci that must be expressed zygotically for the completion of embryonic and imaginal development. While our mutageneses also failed to reveal any lethal complementation groups distal to \(\text{DI}\), in our hands the chromosome comprising \(\text{Tp}(3)\text{bx}^{d10}\) yields a pupal lethal phenotype when heterozygous with either \(\text{Df}(3R)\text{D}^{1BX12}\) or \(\text{Df}(3R)\text{D}^{bx3}\) (deficient for 92A1-92E7, 3) and a lethal phenotype (lethal phase not determined) when heterozygous with \(\text{Df}(3R)\text{Cha}^{M9}\). The same transposition chromosome is viable when heterozygous with either \(\text{Df}(3R)\text{Cha}^{M1}\) or \(\text{Df}(3R)\text{D}^{M2}\). Our data imply the existence of a mutable function that is essential for the completion of imaginal development mapping distal to \(\text{DI}\) within band 92A2. These data, in combination with those of Vassim and Campos-Ortega (1987), suggest an asymmetric distribution of mutable functions proximal and distal to \(\text{DI}\): six or more lethal complementation groups within the 91F6-92A1 interval (nine polytene bands) and one lethal complementation group within the 92A2-92A8,10 interval.
(minimum of seven polytene bands). This is not unprecedented since it is clear from numerous other genetic analyses of small chromosomal intervals that the distribution of lethal complementation groups among polytene bands is widely variable (LEVIEVRE 1974; WOODRUFF and ASHURNBER 1979; NICKLAS and CLINE 1983; CROSBY and MEYEROWITZ 1986).

The ten recessive lethal or semilethal mutations allelic to Dl were mated inter se in all possible pairwise combinations. Although each of the ten fails to complement DfE14, the inter se complementation data indicate that certain allele pairs are partially or fully complementing (Table 2). Moreover, some members of this set of ten Dl mutations are also capable of complementing various members of a set of additional Dl alleles previously isolated in a variety of F1 screens (A. L. TERRY and M. A. T. MUSKAVITCH, unpublished data). This interallelic complementation behavior for alleles within Dl indicates that the locus may be genetically complex (CARLSON 1959; WELSHONS and VON HALLE 1962; LEWIS 1978). A similar suggestion has recently been put forward by VASSIN and CAMPOS-ORTEGA (1987).

This interpretation of our complementation data is clouded, however, by the fact that most alleles that in our hands exhibit intralocus complementation appear to be partial-loss-of-function alleles (see below, Table 2). For example, all the Dl alleles that fail to yield a dominant wing phenotype when heterozygous with a wild-type allele (BE31, BE33-BE37; see below) complement a number of known deficiencies for the Dl locus to variable extents. We are therefore, at present, uncertain whether this apparent interallelic complementation behavior reflects the existence of discrete subfunctions within Dl or results from the production of amounts of a single functional product at a level adequate for survival in some transheterozygotes.

The contention that the observed complementation behavior is indeed interallelic rests on the inference that each mutation analyzed maps within the Dl locus. However, the validity of transheterozygous complementation behavior for assessing allelism among neurogenic gene mutations can be questioned in light of the fact that certain loss-of-function alleles of Dl are lethal in transheterozygous combination with some loss-of-function alleles of E(spl) (VASSIN, VIelmETER and CAMPOS-ORTEGA 1985; S. B. SHEPARD and M. A. T. MUSKAVITCH, unpublished data). We therefore set about localizing each of the ten apparent Dl alleles on the meiotic map of the right arm of the third chromosome.

**Meiotic mapping:** Three zygotic neurogenic loci have been mapped to the right arm of the third chromosome: neu at map position 50.5, Dl at map position 66.2 and E(spl) at map position 89.1 (LINDSLEY and GEELE 1968; LEHMANN et al. 1983). E(spl) has been localized cytotologically to band 96F and our screening procedure should have detected only lethals uncovered by Df(3R)Cha96, the distal breakpoint of which is in 92A2. However, given the complementation behavior in transheterozygotes noted above, we decided to address the possibility that one or more of the ten apparent Dl alleles we have isolated might actually represent mutations at the E(spl) locus.

Balanced stocks, in which the apparent Dl allele is linked to ss (map position 58.5), e+ (map position 70.7) and ro (map position 91.1), were crossed to wild-type Oregon-R virgins (Figure 3). Virgin females heterozygous for a mutation-bearing chromosome and an Oregon-R third chromosome were then mated to ss e+ ro ca males so that recombinant chromosomes could be identified and analyzed. Males carrying nonrecombinant chromosomes or chromosomes representing crossovers in the ss-e interval or the e-ro interval were crossed individually with virgins of a conveniently marked stock, PrITM6B,Dl, which carries the Dl allele CE14 on the balancer chromosome TM6B. This allele fails to complement each of the mutations analyzed (Table 2). Chromosomes carrying any one of these ten mutations will therefore fail to complement DfE14 (i.e., fail to produce Prickly+ claret+ offspring) when mated to PrITM6B,Dl virgin females. For each of the mutations analyzed, apparent nonrecombinant parental chromosomes ss+ e+ ro+ (class E) and ss- e- ro- (class F) complemented DfE14 (class E) or failed to complement this allele (class F), respectively. These data suggest that each mutation tested is closely linked to the ss-ro interval. If this were not the case, we would have observed the loss of the mutation from the parental isogenic ss e+ ro chromosome at some frequency and the acquisition of the mutation by the wild type chromosome at a similar frequency. Recombinant chromosomes representing one class of crossovers in the e-ro interval, ss- e- ro- (class D), failed to complement DfE14; whereas, recombinant chromosomes representing reciprocal crossovers within the same interval, ss- e+ ro- (class C), did complement DfE14. These data are consistent with the localization of each of the ten mutations within the ss-e interval, as expected for Dl alleles. Males carrying chromosomes representing crossovers in the ss-e interval [ss+ e+ ro- (class A) or ss- e- ro+ (class B)] were also crossed individually with PrITM6B,Dl virgin females. The resulting data regarding the cosegregation of each putative Dl allele with ss (class A) or e (class B) permitted calculation of the apparent meiotic map position for each allele with respect to either ss or e. When the two respective values calculated on the basis of data for recombinant chromosomes in each class were averaged for each allele, the ten mutations were found to map at positions 60% to 85% distal from ss within the ss-e interval (meiotic map positions 66 to 69). Dl
Figure 4.—Phenotypes associated with various Delta alleles. Embryonic cuticular phenotypes: A, Wild type (ss $D^R_{E33}$ $e^4$ rol/TM6B); D, weak hypomorph (ss $D^R_{E33}$ $e^4$ rol/Df); G, intermediate hypomorph (ss $D^R_{E33}$ $e^4$ rol ca); J, amorph (ss $D^R_{E33}$ $e^4$ rolDf). Embryonic neural phenotypes: B, wild type (ss $D^R_{E33}$ $e^4$ rol ca/TM6B); E, weak hypomorph (ss $D^R_{E33}$ $e^4$ rol homozygous); H, intermediate hypomorph (ss $D^R_{E33}$ $e^4$ rol ca homozygous); K, amorph (ss $D^R_{E33}$ $e^4$ rol homozygous). Wing phenotypes: C, wild type (ss $e^4$ rol homozygous); F, weak hypomorph (ss $D^R_{E33}$ $e^4$ rol/+); I, intermediate hypomorph (ss $D^R_{E33}$ $e^4$ rol/+); L, amorph (ss $D^R_{E33}$ $e^4$ rol/+). In the genotypes given above, Df is an abbreviation for Df(3R)$D^R_{E33}$ and + is an abbreviation for a third chromosome from the strain Oregon-R carrying a wild-type $Dl$ allele. Tissues and cuticular structures were prepared, mounted and photographed as described in MATERIALS AND METHODS. Embryos are oriented such that anterior is to the left and dorsal is up. Scale bar in panel J represents 250 μm and applies to the left and middle vertical columns; scale bar in panel L represents 750 μm and applies to the right vertical column.

has been mapped meiotically to position 66.2, 7.7 map units distal to ss and 4.5 map units proximal to $e$. The data are therefore consistent with the localization of all ten mutations to the previously defined position of $Dl$. It is clear that none resides in the $e$-ro interval within which E(spl) mutations map (Lehmann et al. 1983).

Relationship between genotype and phenotype:
Four of the ten $Dl$ mutations isolated (BE30, BE32, BE38 and BE39) yield a dominant Delta$^-$ wing phenotype when heterozygous with a wild-type allele while the remaining six $Dl$ mutations have no effect on wing morphology in analogous heterozygotes (Figure 4). Thus, our $F_2$ screen did yield a class of lethal $Dl$ alleles that would not have been acquired in a first generation screen based on the haploabnormal $Dl$ wing phenotype. As we indicated above, it is as yet unclear whether the existence of this class of purely recessive alleles reflects genetic complexity or mere quantitative variation. We have also found that the haploabnormal wing phenotype associated with heterozygosity for $Dl$ alleles cosegregates with the embryonic lethal phenotype for alleles BE30, BE32 and BE38 (appropriate data were not collected for allele BE39).

None of the mutations in the six complementation groups proximal to $Dl$ appears to affect wing morphology when heterozygous with a wild type allele,
nor do alleles representative of each complementation group lead to neural hypertrophy or cuticular defects when homozygous. We therefore infer that the Dl-proximal loci we have identified are functionally, as well as genetically, distinct from Dl.

Examination of the embryonic and imaginal phenotypes associated with each of the ten independent Dl alleles isolated revealed an array of allele-specific effects on epidermal and neural development (Figure 4). Epidermal development was assessed on the basis of cleared cuticle preparations (Hoffmann and Goodman 1987) in mutant hemizygotes and homozygotes, assuming that the structure and amount of cuticle formed faithfully reflect the extent of epidermal development. Neural development was assessed in mutant homozygotes at mid-embryogenesis on the basis of direct immunofluorescence using antibodies against horseradish peroxidase (anti-HRP). Such sera detect epitopes specific to the Drosophila nervous system (Jan and Jan 1982). Adult wing phenotypes were assessed on the basis of light microscopy in homozygotes carrying one wild type allele Dl and one mutated allele. These analyses permitted definition of an allelic series for mutations affecting Dl function.

The mutation DlBE33 represents a weak hypomorphic allele of the locus (the other such allele isolated is BE30). These alleles are considered amorphic since they yield the same phenotypes in homozygotes, hemizygotes and heterozygotes as does a deficiency for the locus (Muller 1932). This class of alleles yields a further reduction in the epidermis (Figure 4J). Only posterodorsal cuticle forms, dorsal hair rows are disrupted and Filzkörper are absent. This further reduction in the epidermis may relate to an increase in the apparent extent of neural tissue in such embryos (Figure 4K). Staining of neural cells appears to extend posteriorly from the procephalic region in comparison to that produced by intermediate hypomorphic alleles. This may represent misrecruitment of cells that would normally form the anterodorsal epidermis into the neural cell population. Intense staining also extends dorsolaterally from the ventral midline to the approximate position of the tracheal placodes. Segmental structure of the CNS is obscured, as in intermediate hypomorphs. Adults heterozygous for amorphic alleles exhibit wing defects of increased severity (Figure 4L). Thickening of L2 is more extensive, laterally and longitudinally, than for intermediate hypomorphs, "deltas" are apparent at the marginal termini of L4 and L5 and disruption of the posterior crossvein occurs.

Comparison of the data regarding embryonic epidermal and neural phenotypes and imaginal epidermal phenotypes leads to three conclusions. First, there is an inverse correlation between the extent of neural hypertrophy and the reduction in the epidermis resulting from Dl loss-of-function mutations (Figure 4). This observation is consistent with the premise that neurogenic genes affect a binary choice between neural and epidermal fates within the ectoderm germ layer (Campos-Ortega 1985) and with previous data regarding allelic variants of Dl and other neurogenic loci (Lehmann et al. 1983). Second, the sensitivity of different regions of the embryo to reduction in Dl function varies with a dorsal-ventral polarity. Thus, the ventral ectoderm is most sensitive to reduction in function since this is the first region to be affected by the mildest Dl mutations (Figure 4D). Alleles with increasingly deleterious effects on Dl function yield successively more lateral (Figure 4G) and dorsolateral (Figure 4J) defects. The most likely mediators of this
differential sensitivity are the dorsal-ventral polarity genes (Anderson and Nüsslein-Volhard 1984). Genetic studies have demonstrated that some members of this gene set are, in fact, epistatic to \(Dl\) (Campos-Ortega 1983). Third, there is a general correlation between the severity of embryonic and imaginal phenotypes associated with \(Dl\) loss-of-function mutations. Alleles exhibiting more severe effects on embryogenesis in homozygotes and hemizygotes yield more severe imaginal phenotypes in heterozygotes (Figure 4). However, mutations exist that do have an impact on embryogenesis, but do not visibly affect wing structure in the above-mentioned allele configurations (compare Figure 4D and Figure 4E with Figure 4F). Therefore, while the sensitivities of embryonic and imaginal ectoderm to reductions in \(Dl\) function vary in parallel, the relative sensitivity thresholds for detectable morphological effects differ for these two stages of development.

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**LITERATURE CITED**


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