The determination of sense organs in *Drosophila*: a search for interacting genes

CHRISTINE DAMBLY-CHAUDIÈRE* and LUC LEYNS

Laboratoire de Génétique, Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-St-Genèse, Belgium

ABSTRACT The determination of sense organs in *Drosophila* requires the concerted action of a battery of genes, several of which have been identified. Previous experiments revealed that flies doubly heterozygous for mutations in two of these genes have a reduced number of sense organs, suggesting the existence of a direct interaction between the corresponding genes and/or their products. We have now used this observation to search for mutations in additional genes that would show similar interactions. We have detected 10 recessive mutations that show a dominant reduction in the number of bristles when simultaneously heterozygous for either *Df(2)J27* or *Df(4)M62f*. Among these mutations, 3 are homozygous viable and show striking defects in their bristle patterns, confirming that the genes thus identified play a role in the patterning of sense organs. We conclude that the «gene dose titration» method (Botas *et al.*, 1982) is an efficient method for identifying interacting genes involved in a common process, provided one can identify a well-defined phenotype to look at, and at least one mutation that alters the process. Our experience suggests that its efficiency should be substantially improved by the use of insertional mutagenesis.

KEY WORDS: *Drosophila, neurogenesis, pattern formation, peripheral nervous system, gene interactions*

Introduction

Morphogenesis involves the accurate determination of cell fate according to cell position. How position impinges on developmental decisions is of particular interest in the case of the nervous system, where many different types of neurons must be reproducibly deployed and interconnected. This problem is difficult to analyze in most nervous systems, however, because of the complexity and limited accessibility of the neural tissue. One exception is the peripheral nervous system of insects, where many sense organs are located on the surface of the body and can therefore be easily observed, and where in addition the pattern of sense organs is often very reproducible, so that departures from normality are readily detected. Two good examples of reproducible patterns have been described in *Drosophila:* in the larva, where a fixed number of sense organs form at precise positions on each body segment (Hertweck, 1931; Campos-Ortega and Hartenstein, 1985; Dambly-Chaudière and Ghysen, 1986), and in the adult, where the arrangement of macrochaetes on the head and thorax is so reproducible that each of them has been given a name. These patterns provide a convenient system for studying the mechanism that ensures that the right sense organ appears at the right position during development.

A number of genetic, developmental and molecular studies in several laboratories has led to the view that the formation of a sense organ is a multistepped process where precision is acquired progressively (reviewed in Ghysen and Dambly-Chaudière, 1989). In a first step, the decision to make a sense organ is taken by clusters of ectodermal cells, the proneural clusters. In a second step, one cell is selected in each cluster to become the sense organ precursor (sensory mother cell, SMC) while the other cells lose their proneural potential and become epidermal. The neural precursor is then specified to produce a certain type of sense organ and consequently divides according to a fixed lineage. These divisions will generate the different components of the sense organ, for example in the case of a bristle, the shaft, the socket, the neuron and the sheath cell.

The emergence of a SMC requires at least two groups of genes. The first group comprises the so-called proneural genes, the best characterized of which are *daughterless* and the four genes of the *achaete-scute* complex (AS-C). The AS-C genes are expressed only in the proneural clusters that will give rise to the SMCs of external sense organs (Romani *et al.*, 1989). In the absence of the AS-C genes, no external sense organ is formed (Dambly-Chaudière and Ghysen, 1987) and the SMCs themselves do not appear (Ghysen and O'Kane, 1989). These results suggest that the local expression of the AS-C genes is the first step in the formation of an external sense organ. The second group of genes involved in SMC formation

Abbreviations used in this paper: AS-C, *achaete-scute* gene complex; SMC, sensory mother cell.

***Address for reprints**: Laboratoire de Génétique, U.L.B. 67, rue des Chevaux 1640 Rhode-St-Genèse, Belgium. FAX: 32.2.650.9744.

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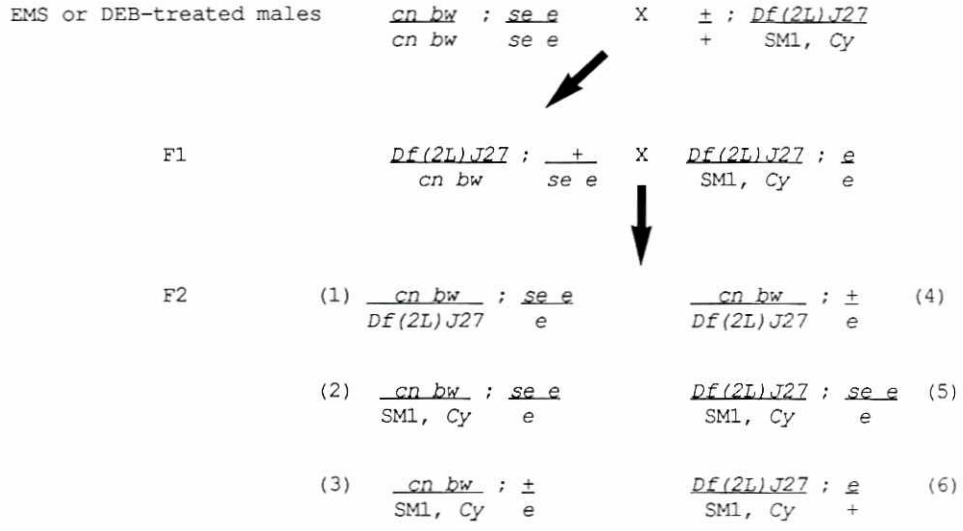

Fig. 1. Scheme of the genetic set-up for the screening of mutations that show a dominant phenotype when combined with *Df(2L)J27*. *In the F2 generation, the analysis of the different categories of progeny answers the following questions:* **(1)** *shows whether the bristle defect in the F1 candidate is hereditary;* **(2)** *shows whether the mutation is a true dominant, or whether it produces a dominant phenotype only in combination with* Df(2L)J27; **(3)** *if the mutation is a true dominant, this class of progeny tells whether the dominant is on chromosome 2 or 3, (4-6) if the mutation is a recessive that produces a pseudo-dominant phenotype in the presence of* Df(2L)J27. *The analysis of these three classes reveals whether the mutation is on chromosome 2, 3 or 4.*

are the «neurogenic» loci. Inactivation of any of these genes leads to a neural hypertrophy (Lehmann *et al.*, 1983) which, in the case of the PNS, is due to the conversion into SMCs of several, or possibly all, cells of the proneural clusters (Simpson, 1990; Goriely *et al.*, 1991). These results suggest that the neurogenic loci are involved in the second step which limits to one the number of proneural cells that can become SMC. Several of these genes are membrane proteins or are potentially involved in signalling systems, suggesting that the second step involves interactions among the cells of each proneural cluster (reviewed in Artavanis-Tsakonas and Simpson, 1991).

What exactly the relation is between the proneural and the neurogenic genes, and how the precise spatial and temporal pattern of expression of the AS-C genes is regulated, is still obscure. In order to answer these questions, and to understand the entire genetic network that controls this early stage of sense organ development, we have attempted to identify other genes that might be involved in this operation. The method we have used is the «gene-dose titration method» based on the idea that changing the gene dosage of two interacting genes may sometimes result in an abnormal phenotype even if changing the dosage of either of the two genes individually has no detectable effect. This method was initially designed by Botas *et al.* (1982) in a search for trans-regulators of genes of the AS-C and BX-C *(bithorax* complex) loci.

The titration method can be used either for testing whether two known genes interact with each other, or to isolate mutations in genes that interact with a known gene. When used to assess potential interactions, the titration method involves the analysis of flies that are doubly heterozygous for mutations in the two genes to be tested (if deletions are used to ensure a complete loss of function of the gene, the flies to be examined are in effect doubly hemizygous). We have previously used this approach to show that adult flies that are simultaneously heterozygous for the deletion *Df(1)260.1*, which removes the AS-C, and for the deletion *Df(2)J27*, which removes *da*, have a reduced number of sensory bristles (Dambly-Chaudière *et al.*, 1988). The existence of a direct interaction between the AS-C and *da* gene products, inferred from these results, has now been amply documented (Murre *et al.*, 1989; Vaessin *et al.*, 1990). We also observed that a third deficiency, *Df(4)M62f*, shows an interaction phenotype with *Df(1)260.1*.

The observation that the double heterozygote AS-C⁻/+; *da*⁻/+ shows a defect in the pattern of sense organs on the notum encouraged us to use the method, and the pseudo-dominant phenotype thus defined, to search for mutations in other genes that would interact with either of the three interacting deletions *Df(1)260.1*, *Df(2)J27*, or *Df(4)M62f*. In our case, due to technical difficulties in using *Df(1)260.1* because of its position on the X chromosome, we have used *Df(2)J27* or *Df(4)M62f* as «tester» deletions.

In order to isolate new mutants, we screened for mutations that give a dominant phenotype in flies that are heterozygous for either *Df(2)J27* or *Df(4)M62f*. The mutagenized flies were crossed with a tester strain containing either deficiency, and the progeny were screened for the presence of dominant phenotypes altering the number of bristles on the adult notum. These will be due either to the occurrence of a true dominant mutation, or to the interaction of a recessive mutation with the tester deficiency. The two possibilities are easily distinguished at the next generation. We recovered a few true dominant mutations, and several recessive mutations which give a dominant phenotype only in the presence of either

TABLE 1

SCREEN WITH *Df(2L)J27*

	EMS	DEB	Total
Number of F1 flies analyzed	2259	2785	5044
F1 flies with a bristle defect	44	70	114
F2 flies with a bristle defect	4	6	10
Dominant mutations	2	1	3
Dominant that shows an interaction		1(C79)	1
Recessive mutations	2	4	6
on chromosome 2	C19	C54, C103	
on chromosome 3	C18	C62, C106	

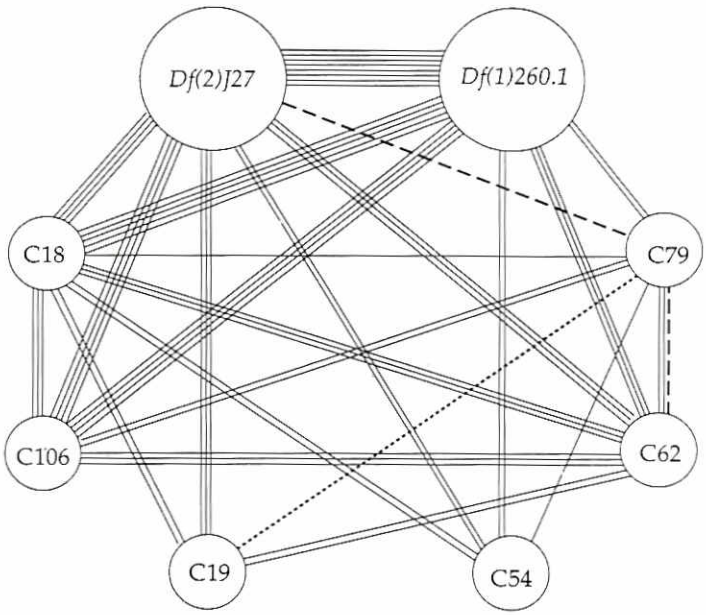

Fig. 2. Network of interactions between *Df(2)J27, Df(1)260.1* and 6 mutations isolated as interacting with *Df(2)J27*. *All possible pairwise combinations between the 8 mutations were analyzed for their bristle phenotype on the head and thorax. In each combination at least 20 flies were examined. Each line indicates the absence of one specific bristle in at least 30% of the cases. Dashed lines reflect the rescue of a bristle usually missing in C79/+ heterozygotes (see text); the dotted line indicates the lethality of the combination.*

deficiency, and which therefore identify new genes presumably involved in an early step of sense organ formation.

Results

Choice of a pseudodominant phenotype.

In a previous screen of about 100 deletions covering together about 30% of the genome, Jan *et al.* (1987) identified several deletions that lead to a massive reduction of the PNS in homozygous embryos. The six most extreme of these deletions were selected for an analysis of potential interactions. All six deletions were combined pairwise; two of the resulting double hemizygotes showed a reproducible reduction in the number of macrochaetes on the notum (Dambly-Chaudière *et al.*, 1988). One of the two combinations involves the deficiencies *Df(1)260.1* and *Df(2)J27*. In this case the interaction was narrowed down to the deletion respectively of the AS-C genes, and of *da*. Embryos homozygous for a deletion of *da* lack all sense organs (Caudy *et al.*, 1988), while deletions of AS-C result in the lack of all external sense organs and of a subset of the internal sense organs (Dambly-Chaudière and Ghysen, 1987). Both *da* and the AS-C genes are known to be involved in the very first step of the formation of sense organs, the determination of the precursor cell (Ghysen and O'Kane, 1989). When heterozygous, both mutations show a wild-type phenotype. The fact that flies doubly heterozygous for *Df(1)260.1* and *Df(2)J27* show a reduction in the number of macrochaetes suggested that this might be a convenient phenotype to screen for new mutations in genes that would interact with either *da* or AS-C.

Search for mutations that show a pseudo-dominant effect when doubly heterozygous with Df(2)J27

The genetic screen used to identify new mutations that interact with *Df(2)J27* is summarized in Fig. 1. Homozygous *cn bw; se e* males fed either EMS or DEB were mated with *Df(2)J27*/SM1 females. We scored the non-SMI male F1 progeny for a dominant phenotype altering the pattern of bristles on the thorax and head. Each F1 candidate was crossed with two *Df(2)J27*/SMI females, and the F2 progeny were examined for the segregation of the mutant phenotype. This allowed us to confirm the mutational origin of the bristle defect, to assess whether the phenotype was a true dominant or a recessive that interacts with *Df(2)J27*, and to determine at the same time which chromosome is affected by the mutation (see Fig. 1). The results of these screens are given in Table 1. The two mutagens, EMS and DEB, give very similar results: for both of them, we recovered interesting mutants at a rate of about one F2 line per 500 screened F1 flies. Of these F2 lines which give a reproducible bristle defect in combination with *Df(2)J27*, 6 are completely recessive mutations, three are dominant mutations, and one (C79) presents both a dominant phenotype and an interaction with *Df(2)J27*. The three dominant mutations have very similar phenotypes and were all shown by complementation experiments to be alleles of the *Hairless* gene, these will not be considered further. Of the six recessive, three are located on chromosome two: C19, C54 and C103, and three are located on chromosome three: C18, C62 and C106. Our crosses were such that no mutation on the X chromosome could be recovered.

We first examined whether these mutations, all of which cause a reproducible defect in the bristle pattern in combination with *Df(2)J27*, also interact with a deficiency for the AS-C genes, *Df(1)260.1*. When combined with *Df(1)260.1*, all but one mutation (C19) gave a dominant phenotype (Fig. 2). The phenotypes differ between different mutations, that is, the subset of bristles that are affected varies from one combination to another. This suggests that the dominant phenotype reflects a direct interaction between the newly identified genes and the AS-C, rather than a cumulative effect of subthreshold defects. Furthermore, for a given mutation, the dominant phenotype is very similar when combined with *Df(1)260.1* or with *Df(2)J27*, suggesting that the corresponding gene interacts in the same manner with both AS-C and *da*. A plausible explanation is that the new gene interacts with the AS-C/*da* heterodimer, suggesting that several of the genes that have been identified in this screen might be targets of the proneural genes.

We also examined whether the new mutations interact with each other, by examining all pair-wise combinations of these mutations in doubly heterozygous flies. The phenotypes of the resulting double heterozygotes are shown in Fig. 2. Many pairwise combinations exhibit defects in the pattern of macrochaetes. The defect varies from one combination to another, as illustrated by the following few examples. One interaction leads to lethality (C1I9 and C79). In another case (C62), the double heterozygotes all have a very similar phenotype, irrespective of which is the second mutation, suggesting that the gene affected by this mutation is specifically required at those few positions. In contrast, the different trans-heterozygotes with C18 have defects at many different positions, indicating that C18 affects a gene generally required for the development of all macrochaetes. Finally the case of the dominant mutation C79 is peculiar in the sense that its phenotype is either enhanced or attenuated in double heterozygotes according to the particular mutation to which it is combined.

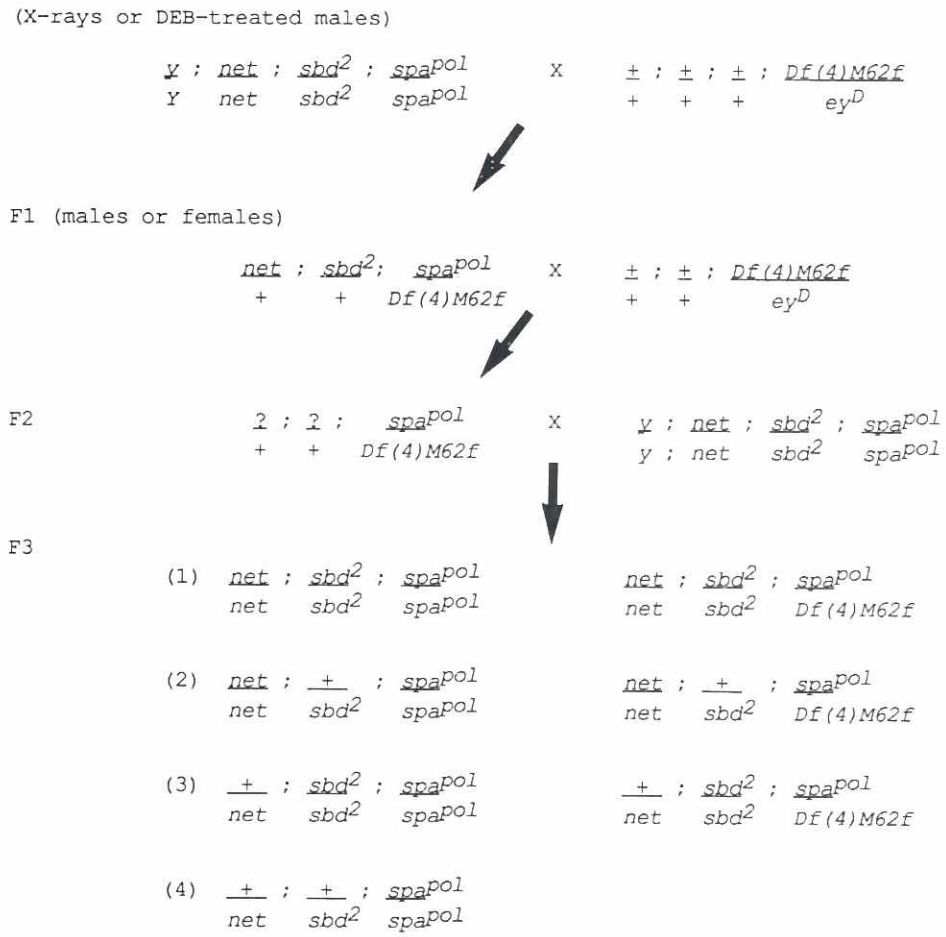

Fig. 3. Scheme of the genetic set-up for the screening of mutations that show a dominant phenotype when combined with *Df(4)M62f*. *The F2 cross was not strictly necessary in the scheme; it was introduced as an intermediate step to assess the heritable character of the defect. The analysis of the different types of F3 flies reveals whether the mutation is a true dominant (class 1), and if so whether it maps to chromosome 2 (class 2), 3 (class 3) or 4 (class 4). If the mutation behaves as dominant only in the presence of* Df(4)M62f *(class 5), then classes 6 and 7 tell us whether it maps to chromosomes 2 or 3.*

Among the 6 recessive mutations uncovered in this screen, one (C62) is homozygous viable. The homozygous phenotype is illustrated in Fig. 4B,G. The medial region of the notum is underdeveloped, resulting in a very reduced notum, and the corresponding bristles (dorsocentrals, posterior post-alars and scutellars) are missing. These bristles are the same as the ones affected in doubly heterozygous conditions, confirming our previous conclusion that this gene is required for proper development at specific positions, rather than in a general step of macrochaete development. Given the morphological defect observed in the homozygous adults, it seems likely that the effect of this gene on patterning extends beyond the generation of sense organs.

Homozygosity for any of the other five mutations leads to embryonic lethality. In all cases the development of the homozygous embryo is blocked before the stage at which the epidermis differentiates. This may be either because the mutation that causes the interaction phenotype has an effect at an early stage of embryogenesis, or because of the presence of additional mutations on the same chromosomes. Whatever the reason, this early defect has made it impossible so far to assess directly the effect of these mutations on the development of larval sense organs.

Analysis of Df(4)M62f

The second combination that led to a reduction in the number of macrochaetes in double heterozygotes involves the deficiencies *Df(1)260.1* and *Df(4)M62f* (Dambly-Chaudière *et al.*, 1988). We have examined which gene(s) of the latter deficiency are responsible for the interaction phenotype.

We tested alleles of the different genes known to be deleted in *Df(4)M62f* in doubly heterozygous combination with *Df(1)260.1*. We found an interaction with representatives of two lethal complementation groups, *1(4)13* and *1(4)17*, which define a complex locus previously identified by virtue of the dominant mutations, Ce^2 and ci^D. Recessive mutations in both complementation groups result in the same segmentation phenotype (Wieschaus *et al.*, 1984; Orenic *et al.*, 1987), and both apparently correspond to a single transcription unit coding for a zinc-finger protein (Orenic *et al.*, 1990). Thus the interacting phenotype can be mostly, and possibly uniquely, ascribed to the deletion of this complex locus. Interestingly, the transcription unit is expressed in the anterior compartment of the wing disc (Eaton and Kornberg, 1990), where all the macrochaetes originate. It is therefore possible that the product(s) of this locus are required for the proper activation of the AS-C genes in the anterior compartment, and that in this case the existence of an interaction reflects a direct requirement of AS-C for a spatially restricted activating factor.

Search for mutations that show a pseudo-dominant effect when doubly heterozygous for Df(4)M62f

Mutations that interact with *Df(4)M62f* were identified according to the scheme described in Fig. 3. We mutagenized *y; net; sbd²; spa^pol* homozygous males with either X rays or DEB and mated them with

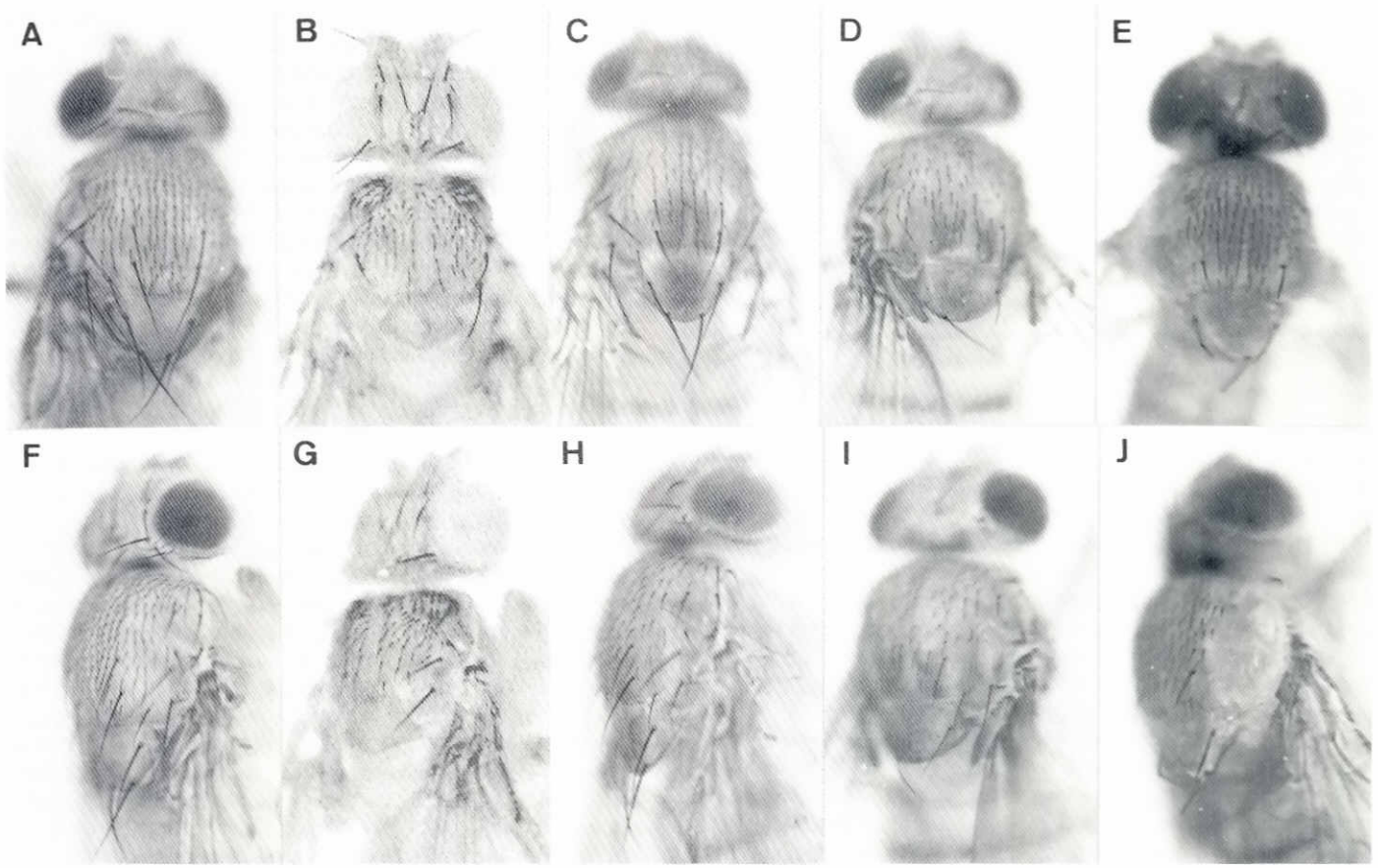

Fig. 4. The pattern of bristles in wild type and mutant flies. (A,F) *wild type;* **(B,G)** *C62 homozygote showing the disappearance of the medial region of the notum;* **(C,H)** *L9* (N^{Nu}) *heterozygote showing a reduction in the number of microchaetae;* **(D,I)** *L12* (deg) *homozygote showing a reduction of both macro- and microchaetae on the head and notum,* **(E,J)** *L19* (iro) *homozygote where the bristles are completely missing on the lateral regions of the notum.*

Df(4)M62f/ey^{D} females. Their progeny were screened for non-*ey*^{D} flies presenting a bristle defect on the notum or head. We assessed whether the defect was heritable by crossing each F1 candidate with *Df(4)M62f/ey*^{D} females. The F2 candidates were then tested for the dominant or recessive character of the mutation and its chromosomal location by crossing them with *y; net; sbd*^{2}*; spa*^{pol} homozygous females, as explained in Fig. 3.

The results of these screens are shown in Table 2. The frequency of mutations recovered with *Df(4)M62f* appears higher than that obtained with *Df(2)J27* (1 in 130 instead of 1 in 500 mutagenized chromosomes). This may, however, reflect differences in the handling of the candidates rather than a real difference between the two screening set-ups. For example, some of the mutations retained in the *Df(4)M62f* screen presented an interesting phenotype but with a weak penetrance, and therefore were ultimately lost or discarded. We finally obtained 8 balanced mutations displaying a dominant bristle defect phenotype in combination with *Df(4)M62f*.

Two of the three dominant mutations were *H* alleles and were not further considered. The third mutation, L9, maps on the X chromosome. The dominant phenotype of L9 consists of a 50% reduction in the number of microchaetes on the notum (Fig. 4C,H). In addition, the mutation is recessive lethal: L9/L9 females or L9/Y males die during the larval stage. Meiotic mapping showed that L9 is localized between *sc* (1-0) and *cho* (1-5), close to the gene *Notch (N)*. A complementation test based on the recessive lethality indicates that L9 is an allele of *N*, since L9/N heterozygotes are lethal. Likewise the presence of a duplication for N^{+} on an autosome rescues the lethality of L9/Y males. Interestingly the dominant phenotype of this mutation is limited to the reduction in the number of bristles, without the concomitant effects on the wing such as the wing nicks or vein gaps shown by the known dominant *N* mutations. We therefore called this allele Notch-unlike (N^{Nul}). The difference in phenotype suggests that N^{Nul} defines a new class of alleles which specifically alter the bristle-spacing function of *N*.

One mutation (L12) has a weak dominant phenotype (two bristles are missing in 20% of the flies) which is enhanced in the presence of *Df(4)M62f*. This mutation is homozygous viable and results in a marked adult phenotype: about 70% of the macrochaetes and 45% of the microchaetes of the head and thorax are absent (Fig. 4D,I). Because of this marked reduction of hairs, the mutation has been named *dégarni (deg)*. All bristles are equally affected, irrespective of their position. The defect is not limited to the head and thorax: the leg bristles are also reduced in number, and other sense organs are affected as well, in particular the campaniform sensilla

TABLE 2

SCREEN WITH *Df(4)M62f*

	X-R	DEB	Total
Number of F1 flies analyzed	4074	2376	7350
F1 flies with a bristle defect	102	139	241
F2 flies with a bristle defect	20	19	39
Number of balanced lines	3	5	8
Dominant mutations	2	1 (L9)	3
Dominant that shows an interaction		1 (L12)	
Recessive mutations	1	3	4
on chromosome 3	L19	L8, L10, L11	

of the wing, and the chemosensory organs of the proboscis. Thus *deg* seems to be involved in a step that is common to the formation of most sense organs of the head and thorax. The bristle pattern on the abdomen, however, is unaffected.

When tested for its interaction with the deletion *Df(2)J27*, *deg* showed a phenotype even stronger than that of the homozygous flies, suggesting that *deg* maps within the region deleted by *Df(2)J27*, and therefore that *deg/Df(2)J27* are in effect hemizygous for *deg*. The gene affected by the *deg* mutation is distinct from *da*, however, because *deg/da* heterozygotes are completely normal and fertile. We examined the progeny of *deg/da* females to assess the frequency of recombination between the two mutations. We did not find a single da^+ deg^+ recombinant among 14,500 flies examined, suggesting that *deg is* very close to *da, or* that recombination is locally depressed. The examination of salivary gland chromosomes did not reveal any rearrangement, and therefore we conclude that *deg* defines a gene mapping in the region defined by *Df(2)J27*, and distinct from *da*.

Two of the four recessive mutations that show an interaction with *Df(4)M62f* are allelic. One of the two (L8) is recessive lethal but the other (L19) is viable and shows the striking phenotype shown in Fig. 4E,J, where only a median strip of hairs remains on the notum. The L8/L19 heterozygote shows exactly the same phenotype, demonstrating the allelism of the two mutations. The affected gene was called *iroquois (iro)*, by analogy with the American Indian tribe that used a similar haircut. In some countries this haircut is ascribed to the Mohawk nation; we have not been able to resolve the origin of the confusion, nor to determine with certainty which of the two names is the most appropriate. The characterization of the *iro* mutations will be published elsewhere.

The other two mutations that show an interaction with *Df(4)M62f*, L10 and L11, are both recessive lethals and map to the third chromosome. The L10/L11 heterozygote is also lethal, suggesting either that L10 and L11 define two genes that interact very strongly, or that the two mutations are alleles of the same gene and therefore do not complement each other. The fact that both mutations show nearly identical phenotypes in combination with *Df(4)M62f*, with *Df(1)260.1* or with *Df(2)J27*, suggest that they are indeed alleles of the same gene.

Discussion

The «gene dose titration method» (Botas *et al.*, 1982) has been used with success to identify new genes involved in different multigenic processes: sex determination (Belote *et al.*, 1985), sense organ formation (Botas *et al.*, 1982), and maternal contribution to the antero-posterior organization of the embryo (Tricoire, 1988). The titration method presents several practical and theoretical advantages over the more usual method of analyzing individuals homozygous for newly generated mutations. First, it is possible to score a mutation as a F1 heterozygote, instead of having to score F2 homozygotes (and therefore having to build a line for each mutagenized chromosome). Second, in the case of genes that are essential at several stages of development, homozygous mutants will generally be blocked at the first stage, making it difficult to assess additional functions of the gene at later stages.

Our results demonstrate that the gene titration method is a powerful and valuable method for a systematic search for genes involved in the determination of sense organs. We were able to isolate several mutations that show an interaction with either *Df(2)J27* or *Df(4)M62f*, two deficiencies known to alter the development of the PNS, in doubly heterozygous flies. In addition three of these mutations are homozygous viable and result in striking defects in bristle patterning in homozygous adults. This result indicates that the mutations that have been isolated do indeed alter the development of the normal pattern of sense organs. The larval PNS of these mutants is nearly normal (a few sense organs are missing in about 10% of the larvae, data not shown). This lack of larval phenotype may be due to a specific requirement of the corresponding genes for the generation of the adult, and not of the larval, sense organs. Alternatively it may be that the genes are involved in sense organ determination at both stages, but that the embryonic defect is rescued by the presence of gene product provided by the mother. For all three mutants the homozygous flies are viable but sterile, so that all the progeny come from heterozygous mothers. The distinction between the two possibilities would therefore require the generation of homozygous germ line cells.

In most cases we have recovered only one allele per gene, suggesting that we are far from saturation. A crude estimate based on the Poisson distribution of single and double hits indicates that there might be as many as 18-20 genes that show a genetic interaction with *Df(2)J27* and/or *Df(4)M62f*. This number is high, but it should be noted that it may include both regulators and targets of *da* and the $Ce\text{-}ci^D$ complex. Furthermore, since *da* and the AS-C genes themselves interact directly, it may be that genes that interact with AS-C would also show an interaction with *da*.

While our search for new genes involved in the determination of sense organs has been fruitful, a major limitation has been that the lethal mutations turned out to be difficult to map. This is because, in order to map a mutation, one needs a clear-cut associated phenotype so that one can assess whether or not each recombinant chromosome carries the mutation. For most of the lethal mutations that we isolated we could not identify a clear-cut defect in the pattern of larval sense organs. In some cases we could not assess the PNS phenotype because the mutation prevented the homozygous embryo from reaching the relatively late embryonic stage where the larval sense organs have fully differentiated and can be observed in cuticle preparations. This may result from an alteration of some early developmental step either due to the interacting mutation, or due to another mutation induced on the same chromosome. In other cases the homozygous embryos did reach the end of embryogenesis, but the sense organs were largely normal. This, of course, does not imply that the mutation is of no interest: the lack of phenotype could result from a rescue by maternal product, as

mentioned above. Whatever the reason for the lack of obvious phenotype in the homozygous embryos, it greatly complicated the mapping of the mutations. The mapping of lethality itself is of little use, since a substantial fraction of the mutant chromosomes will carry more than one lethal mutations. An alternative procedure would have been to map the mutations by relying on the interaction, that is, to map them in heterozygous background. This method turned out to be impractical because in most cases the interaction is relatively sensitive to differences in genetic background, and the analysis of the different classes of recombinants did not give unambiguous responses. One way to circumvent this problem is to use insertional mutagenesis with the P element, which can then be easily mapped by *in situ* hybridization on polytene chromosomes.

Materials and Methods

Strains

The deficiencies and genetic markers are described in Lindsley and Grell (1968) and obtained from the stock centers at Caltech, Bowling Green and Umea except *1(4)13* and *1(4)17* which were described in Orenic *et al.*, (1987), and obtained from T. Orenic. The *N* alleles are N^{55e11} and $Df(1)N^{8}$, and DpN^{+} is $Dp(1;2)w^{+51b7}$.

Mutagenesis

The crosses used to generate new mutations are described in Figs. 1 and 3. Mutations were induced in males either by X-ray irradiation (4000R at 150 kV, 25mA, 2mm Al filter) or by feeding them ethylmethane sulfonate (EMS, 0.025 M in 1% sucrose) or diepoxibutane (DEB, 0.006M in 1% sucrose). These doses result in the induction of about 3.2 lethal mutation per haploid genome.

Test of interactions

The existence of an interaction between two mutations was examined by scoring the bristle phenotype of doubly heterozygous adult flies. Balanced stocks were crossed and the phenotype of the F1 double heterozygote was assessed. In cases where a defect was noted, we repeated the experiment after first outcrossing the two mutations (crossing the two balanced stocks with wild type flies) in order to reduce possible differences in genetic background or modifiers. We then scored the phenotype of the F2 progeny. The results given in this paper always correspond to the phenotype after outcrossing.

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