Scalloped wings Is the *Lucilia cuprina Notch* Homologue and a Candidate for the *Modifier* of Fitness and Asymmetry of Diazinon Resistance

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ABSTRACT

The *Scalloped wings* (*Scl*) gene of the Australian sheep blowfly, *Lucilia cuprina*, is shown to be the homologue of the *Drosophila melanogaster Notch* gene by comparison at the DNA sequence and genetic levels. A *L. cuprina* genomic fragment, which shows strong identity with the *Notch* (*N*) gene at the molecular level, hybridizes to the location of the *Scl* gene on polytene chromosomes. The two genes are functionally homologous; the dominant and recessive *Notch*-like phenotypes produced by mutations in the *Scl* gene allow these alleles to be classed as *N*-like or *Abruptex*-like. The *Scl* gene is under investigation as a candidate for the fitness and asymmetry *Modifier* (*M*) of diazinon resistance. We show that *M* affects the penetrance of wing and bristle phenotypes associated with two *Scl* alleles in a manner consistent with the *M* being an allele of *Scl*. In addition, we report a phenotypic interaction between the diazinon-resistance mutation, *Rop-1*, and the same alleles of *Scl*. We propose that the product of *Rop-1*, an esterase, may be involved in cell adhesion in developmental processes involving the *Scl* gene product.

THE problematic occurrence of resistance to insecticides in target and nontarget species provides fortuitous case studies for evolutionary biologists (ROUSH and MCKENZIE 1987; MALLET 1989; ROUSH and TABASHNIK 1990). In these situations the selective agent, the insecticide, is known and the significance of changes in susceptible or resistance allele frequency is explicable in terms of the resulting phenotype. For these reasons it is possible to ask detailed questions about the evolution of insecticide resistance. A well documented case is the evolution of resistance to the organophosphorous (OP) insecticide diazinon in the Australian sheep blowfly, *Lucilia cuprina*. Allelic substitution at a single locus (*Rop-1*), which encodes the carboxylesterase E3, is responsible for resistance (HUGHES and RAFTOS 1985; RUSSELL *et al.* 1990; PARKER *et al.* 1991). In the absence of insecticide, the relative fitness of the resistant flies is reduced in comparison with susceptible flies (MCKENZIE *et al.* 1982). In addition, the resistant flies have increased levels of asymmetry, a measure of differences between the right and left sides of a bilaterally symmetrical organism (CLARKE and MCKENZIE 1987; MCKENZIE and CLARKE 1988; MCKENZIE 1993). These fitness and asymmetry effects are proposed to be the result of developmental instability caused by the introduction of the new resistance allele into a genome coadapted for the susceptible allele (CLARKE and MCKENZIE 1987; MCKENZIE and CLARKE 1988).

Continuing selection with diazinon brought the resistance allele frequency close to fixation and provided the necessary conditions for coadaptation of the resistant genome (ROUSH and MCKENZIE 1987). Mutation of a second gene, *Modifier* (*M*), resulted in a dominant increase of the fitness of resistant flies such that, in the absence of diazinon, susceptible and resistant flies had equal fitness (MCKENZIE *et al.* 1982; MCKENZIE and GAME 1987). Coincident with the change in fitness is a decrease in the level of asymmetry of resistant flies, returning asymmetry to the level of susceptible flies (CLARKE and MCKENZIE 1987; MCKENZIE and CLARKE 1988).

The modifier of fitness and asymmetry maps genetically to the region of the *Scalloped wings* locus, a gene proposed to be the homologue of the *Drosophila melanogaster Notch* gene (FOSTER *et al.* 1981). This connection between *Notch* and *Scl* was originally made on the basis of similar adult wing phenotypes and recessive lethality (MADDERN *et al.* 1986) and is further supported by conservation of linkage groups such that both genes map close to the respective *white* genes of *D. melanogaster* and *L. cuprina* (FOSTER *et al.* 1981; WELLER and FOSTER 1993).

The well-characterized *Notch* gene of *D. melanogaster* has a role in the determination of cell fate throughout development in a variety of tissues (reviewed by ARTAVANIS-TSAKONAS *et al.* 1991; FORTINI and ARTAVANIS-TSAKONAS 1993; MUSKAVITCH 1994). The *Notch* gene product (Notch) is a transmembrane protein thought to

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mediate a cell-cell signal through protein-protein interactions at its extracellular and intracellular domains (FEHON *et al.* 1990; REBAY *et al.* 1991; LIEBER *et al.* 1992; DIEDERICH *et al.* 1994). An example of *Notch* function occurs during embryogenesis where Notch is required in the neuroectoderm to allow undetermined cells to adopt either a neural or epidermal fate. Loss of function of the *Notch* gene in the neuroectoderm results in the formation of an excess of neural precursor cells at the expense of epidermal precursors (POULSON 1950; LEHMANN *et al.* 1983). This is known as the "neurogenic" phenotype, a phenotype that is common to mutations in any of the neurogenic genes, a group of genes that appear to act in many or all of the developmental pathways involving *Notch* (LEHMANN *et al.* 1983).

The genetics of the *Notch* locus is complex, there are multiple allele classes that display pleiotropic combinations of dominant and/or recessive adult phenotypes. There are two classes with dominant adult phenotypes, the *Notch* (*N*) alleles, which are hypomorphic mutations, and the hypermorphic *Abruptex* (*Ax*) alleles (LINDSLEY and ZIMM 1992). In addition to the recessive embryonic lethality associated with the neurogenic phenotype described above, the *N* alleles display dominant adult wing notching, wing vein thickening and bristle abnormalities (LINDSLEY and ZIMM 1992). The *Ax* alleles show dominant wing vein gaps and bristle loss. These *Ax* alleles can themselves be divided into three classes, those that are recessive lethal (*l-Ax*) and two classes that either enhance, (*E*(*N*)) or suppress (*Su*(*N*)) the wing notching phenotype of a *N* allele when in heteroallelic combination (FOSTER 1975; PORTIN 1975; LINDSLEY and ZIMM 1992).

Here we examine the relationship between *Scl* and *Notch* at molecular, phenotypic and genetic levels and show that the two genes are homologous. A portion of the *Scl* gene is cloned by hybridization at low stringency using a fragment of the *Notch* gene as a probe. Comparison of the deduced gene products of the two genes reveals very high conservation. The genetic and phenotypic similarities between *Notch* and *Scl* are investigated by analysis of the recessive lethal and dominant adult phenotypes of loss-of-function *Scl* mutations and by isolation and characterization of two *Abruptex*-like mutations of *Scl*. These studies reveal functional homology between *Notch* and *Scl*. We also investigate the possibility that the modifier of fitness and asymmetry of diazinon resistance is an allele of the *Scl* gene. Two incompletely penetrant alleles of *Scl* are used as a sensitive test for interactions between *M* and *Scl* and between *Rop-1* and *Scl*. These experiments support the allelism of *M* with *Scl*. Based on the functional homology between *Notch* and *Scl* we propose a mechanism for the observed developmental effects of the *Rop-1* mutation and the suppression of those effects by *M*.

MATERIALS AND METHODS

Strains used: All stocks were maintained at 27 ± 1° on standard laboratory medium unless otherwise specified. Six mutations of *Scl* used in this study, Scl^1, $T(3:6)\,Scl^2$, Scl^3, Scl^4, $Df(3)\,w\text{-}Scl^2$ and $Df(3)\,w\text{-}Scl^3$, were induced by gamma irradiation and kindly supplied by G. FOSTER and G. WELLER (CSIRO Entomology, Canberra). The Scl^5 mutation was induced with EMS previously in this laboratory. In the course of this study the Scl^{Ax1} and Scl^{Ax2} alleles were EMS-induced and the Scl^6 and Scl^7 mutations were induced by gamma irradiation.

The *In(3LR) 7 + 10, Scl^1 ru* strain carries both the Scl^1 allele and the *rusty body* (*ru*) mutation on a doubly inverted chromosome *III* where the inversions overlap (FOSTER *et al.* 1991).

Two mutations of the *white eyes* gene, *w* and w^m (*white mustard eyes*) (MADDERN *et al.* 1986), were used in mutagenesis, mapping or as chromosome markers in recessive lethality or complementation crosses.

Strains homozygous for the *ru* mutation or carrying a multiply-marked chromosome *III* [*crooked bristles* (*ck*), w^m and *ru* (MADDERN *et al.* 1986)] over the $Df(3)\,w\text{-}Scl^3$ chromosome were used in mutagenesis experiments.

Other strains used for testing combinations of *Modifier* and *Scl* alleles were doubly homozygous for *Modifier* (*M*) or wild-type (+) and diazinon resistance (*Rop-1*) or susceptible (+) alleles (*M/M*; +/+, +/+; +/+, *M/M*; *Rop-1/Rop-1* and +/+; *Rop-1/Rop-1*) in comparable genetic backgrounds. The origin of these strains is described in MCKENZIE and GAME (1987) and MCKENZIE and CLARKE (1988).

Molecular biology: A standard wild-type *L. cuprina* genomic library (gift from TONY HOWELLS, Biochemistry Department, the Australian National University) in λgt11 was screened for *Notch*-hybridizing clones. Plaque lifts were hybridized with the ^{32}P-α-dATP nick translated *Notch* probe at 42° overnight in a solution of 30% formamide, 6× SSC, 5× Denhardts, 0.5% SDS, 200 pg/ml salmon sperm DNA. Filters were washed in 2× SSC, 0.1% SDS at 65° for 1 hr and films exposed at −70°.

Restriction fragments were subcloned into pBlueScript (pBSK$^+$; Stratagene) for DNA sequencing. The double-stranded templates were amplified on a DNA Thermal Cycler (Perkin Elmer Cetus) with T3 or T7 dye primers (Applied Biosystems), according to the instructions of the manufacturers. Sequencing gels were run and the data were read by an ABI 373A automated sequencer (Applied Biosystems). Sequence analysis was performed using the SeqEd v1.0.3 program (Applied Biosystems).

Cytology: Trichogen cell polytene chromosomes were prepared from pupae whose parents were heterozygous (*Scl*/+) for each *Scl* allele. In the case of Scl^{Ax1}, Scl^{Ax2}, Scl^6 and Scl^7 the parents were *Scl* +/+ *w* so that the *white eyed* non-*Scalloped* progeny could be excluded as some pigmentation of the eye is apparent in the pupae at the time of dissection. Chromosome preparation methods follow those of FOSTER *et al.* (1976) and BEDO (1982). Chromosome banding patterns were examined with phase contrast microscopy and compared with the maps of FOSTER *et al.* (1980b).

***In situ* hybridization to polytene chromosomes:** The hybridization procedure followed the nonradioactive protocol of ENGELS *et al.* (1986) with modifications to the chromosome denaturation step suggested by BEDO and HOWELLS (1987). The same chromosome spreads were photographed before and after *in situ* hybridization; this allowed the position of any hybridization to be analyzed accurately as the morphology of a chromosome was more defined before the hybridization process.

Wing preparation: Wings were cut from 1–2 day old flies, dehydrated briefly in 70 and 100% ethanol then mounted in Euparal (GBI Labs), covered with a glass coverslip and flattened with light pressure.

Scanning electron microscopy: Flies to be examined by

scanning electron microscopy were desiccated at room temperature with minimal handling. After mounting on stubs with a silver glue, the flies were coated with 140Å of gold and examined at a magnification of ×302–890 with a Philips SEM505 scanning electron microscope.

Embryo fixation: Embryos (7 ± 0.5 hr old at 27°) were fixed and devitellinized according to the paraformaldehyde fixation method of TAUTZ and PFEIFLE (1989). Subsequent anti-horse radish peroxidase (HRP) staining was according to steps 12–24 of protocol 96 of ASHBURNER (1989). The stained embryos were mounted in Pro-Cure 812 (Probing and Structure) according to protocol 96 of ASHBURNER (1989). The nature of the homozygous *Scl* lethality was investigated by analyzing embryonic progeny from parents heterozygous for each *Scl* allele such that one in four embryos were expected to be homozygous for the mutation at *Scl*.

Cuticle preparation: Cuticles were prepared from embryos (11.75 ± 0.25-hr old at 27°) by treatment with glycerol:glacial acetic acid (1:4) and mounting in Hoyer's Mountant according to the method of WIESCHAUS and NÜSSLEIN-VOLHARD (1986).

Mutagenesis: The method for EMS mutagenesis followed that of SMYTH *et al.* (1992). Treated males were mated with *ck* w^m *ru*/+*Df*(*3*) *w-Scl*^{3} + virgin females. Mutagenesis using gamma irradiation was performed on 5-day-old males of the *ru*/*ru* genotype using an Eldorado 6 ^{60}Co source. A dose of 2800 rad was administered at 184 rad/min. Mutagenized males were crossed with *w*/*w* virgin females. For both types of mutagenesis, putative *Scl* mutants were identified following visual screening of the F_1 progeny.

Testing *Scl* alleles for recessive lethality: Each of the preexisting *Scl* alleles were tested for recessive lethality using the cross diagrammed in Figure 1. The mutation *Scl*^{Ax2} was tested for lethality in a similar manner but utilized the more closely linked w^m mutation in place of the *ru* marker. Under this crossing regime, if a *Scl* allele is recessive viable, then the *Scl*/*Scl* homozygotes can be identified as *ru* homozygotes (*Scl ru*/*Scl ru*). Thus an absence of *rusty Scalloped* flies would be an indication of recessive lethality.

Complementation tests with *Scl*^{1}: The crosses shown in Figure 1 utilized a multiply-inverted chromosome *III* balancer, *In*(*3LR*)*7+10*, which carries the *Scl*^{1} allele and a mutation in *ru* (FOSTER *et al.* 1991). The inversions include the *ru* locus but not the *Scl* region. However, this balancer chromosome has been shown to significantly reduce the frequency of viable crossovers in the interval between *Scl* and *ru* (FOSTER *et al.* 1991). For each *Scl* allele tested, heterozygous male flies were mated with females bearing the double-inversion chromosome. Recombination occurs at an extremely low frequency in male *L. cuprina* (FOSTER *et al.* 1980a), therefore recombination should be virtually eliminated between *Scl* and *ru* ensuring that only *Scl* homozygotes could be homozygous for *ru*.

Temperature shift: Temperature-sensitive periods were determined by raising developing flies at 20, 25 and 27°. For each temperature, white pre-pupae (13–15 days old at 20°, 7–9 days old at 25° and 6–8 days old at 27°) were either kept at that initial temperature or transferred to the alternative temperatures.

***Scl*/*Modifier* interactions:** The effect of the modifier on the phenotype and asymmetry associated with *Scl*^{5} and *Scl*^{Ax1} was determined by performing crosses in pairs where each *Scl* allele was crossed to *Modifier* (*M*/*M*) and non-*Modifier* (+/+) strains in either a susceptible (+/+) or resistant (*Rop-1*/*Rop-1*) background. Comparisons can then be made between the proportion of progeny expressing the *Scl* phenotype to those which do not, in the presence or absence of *M*. For instance, *Scl*^{5}/+;+/+ flies were crossed to *M*/*M*;+/+ and +/+;+/+ strains at the same time so that the progeny developed under similar environmental conditions (the genetic notation used assumes that the modifier is an allele of *Scl*). The genotypes of the parents and their progeny were not revealed to the scorer to prevent bias. The effect of *M* on *Scl*^{4} phenotype and asymmetry was also examined but only in a susceptible background using similar pairs of crosses.

Comparison between the paired crosses allows an examination of the effect of the *Rop-1* allele, if any, on the expression of the *Scl* phenotype. Differences in the proportion of flies expressing the *Scl* phenotype to those which do not can be attributed to the presence or absence of the resistance allele in flies with an otherwise identical genotype.

Asymmetry: The methods followed those of CLARKE and MCKENZIE (1987) and MCKENZIE and CLARKE (1988). Asymmetry of a fly was estimated from the absolute difference in bristle count between the left and right sides of the frontal head stripe, the outer wing margin and the R_{4+5} wing vein. The asymmetry of at least 20 flies was scored to generate a mean asymmetry value for each genotypic combination. In crosses involving *Scl*^{5}, asymmetry was measured for 20 *Scl*^{5}/+ flies each for the two phenotypic classes, expression and nonexpression of wing notching.

RESULTS

Molecular analysis of the *Scl* gene: A positive phage clone, designated λLcT1, was isolated from the genomic library using a 2-kb *Drosophila Notch Bam*HI fragment that contains the cdc10/ankyrin repeats (WHARTON *et al.* 1985). A *Cla* I fragment was subcloned (subclone C4) and the sequence of the 2674-bp insert was determined (Genbank Accession No U35001). Alignment of the DNA sequence and the inferred amino acid sequence with the *Notch* gene indicated the cloned *L. cuprina* DNA fragment is homologous to a part of exon E of *Notch* (Figures 2 and 3; WHARTON *et al.* 1985; KIDD *et al.* 1986). The nucleotide sequences are 62.8% identical and the inferred amino acid sequences share 69.0% identity in the homologous regions of the two genes.

With reference to the predicted Notch gene product, the cloned *L. cuprina* region includes the end of the EGF-like repeats (half of EGF35 and complete EGF36), three Notch/Lin 12 repeats, a transmembrane region and six cdc10/ankyrin repeats. These functional domains are in the same order as in the *Notch* gene. There are obvious differences in molecular conservation between the domains. In the extracellular region of the proteins, the amino acid sequences of the EGF-like repeats and Notch/Lin 12 repeats, which may function as receptors for intercellular signals (REBAY *et al.* 1991), are 74.1 and 73.5% identical, respectively (Figure 3). However, in the intracellular region, the six cdc10/ankyrin repeats are highly conserved. Overall 94.9% of the amino acids of the repeats are identical. The last four repeats, in particular, have 133 consecutive identical amino acids, suggesting a strong functional constraint. In Drosophila these repeats have been shown to bind the deltex and suppressor of hairless proteins thus mediating signal transduction (DIEDERICH *et al.*

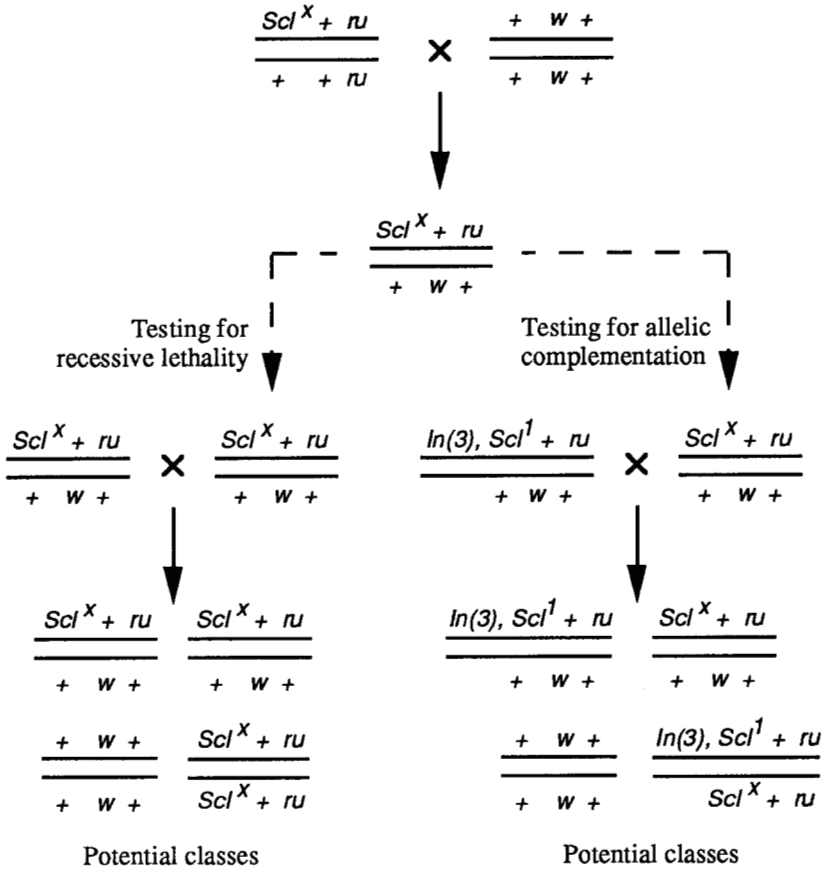

FIGURE 1.—The genetic crosses used to generate flies with each *Scl* allele (Scl^X) in *cis* to *rusty* (*ru*) and in *trans* to *white* (*w*). Once generated, flies from the same generation were crossed either to their siblings with the same genotype as a test of recessive lethality or crossed to the strain carrying Scl^1 on a double-inversion balancer chromosome.

1994; ARTAVANIS-TSAKONAS *et al.* 1995). It has been demonstrated that this region is essential for Notch function (LIEBER *et al.* 1993; REBAY *et al.* 1993). In contrast, in the regions between the identified functional domains sequences are more diverse between *Scl* and *Notch*. For example, the region between the Notch/Lin12 and cdc10/ankyrin repeats has 59.5% identity and the region after the cdc10/ankyrin repeats, 53.3%.

Cytological localization of the *Scl* gene: On the basis of their tight genetic linkage (0.2–1.2 map units; FOSTER *et al.* 1991; WELLER and FOSTER 1993) the *Scl* and *white* genes should be located within a small cytological region. The cytological location of the *white* gene has been identified (Figure 4A) (BEDO and HOWELLS 1987). The location of the *Scl* gene (Figure 4A) has been proposed as the band distal to the location of the *white* gene on the basis of a small cytological deletion associated with the Scl^1 mutation (FOSTER *et al.* 1991), although this conflicts with the translocation breakpoint associated with $T(3:6)\,Scl^2$ (BEDO and HOWELLS 1987).

In light of the conflicting evidence for the position of the *Scl* gene, we examined polytene chromosomes from a number of *Scl* mutants, including two newly induced with gamma radiation (Scl^6 and Scl^7). The mutations Scl^3, Scl^4, Scl^5 and Scl^7 display no abnormal cytology in the proposed region of the *Scl* locus (data not shown). Data from the remaining *Scl* mutations agree with FOSTER *et al.* (1991), positioning the locus at the minor band distal to the *white* band in region 23A (Figure 4). The one exception remains the translocation mutation $T(3:6)\,Scl^2$, which has its breakpoint proximal to the *white* band, not distal. It is possible that the *Scalloped* phenotype associated with $T(3:6)\,Scl^2$ is due to a position effect as a result of the translocation rather than a physical break in the gene.

The *L. cuprina Notch*-homologous genomic fragment, λLcT1, was *in situ* hybridized to polytene chromosomes from pupal trichogen bristle cells. This clone hybridizes to the proposed position of the *Scl* gene in region 23A (Figure 5), providing good evidence that this *Notch*-homologous fragment derives from the *Scl* gene.

Adult phenotypes associated with mutations of *Scl*: It is evident that the adult phenotypes of the *Scl* mutations, Scl^1, $T(3:6)\,Scl^2$, Scl^3, Scl^4, Scl^5, Scl^6 and Scl^7, are similar to those displayed by the hypomorphic *N* alleles of the *Notch* gene (MADDERN *et al.* 1986; LINDSLEY and ZIMM 1992). Flies heterozygous for any of these *Scl* mutations display dominant notching of the trailing and leading wing margins, thickening of wing veins and bristle abnormalities (Figures 6 and 7). Variation in expressivity of the wing notching phenotype is associated with each allele. Despite this variation it is possible to order mutations in an allelic series based on the severity of wing notching such that weak and strong notching phenotypes can generally be associated with

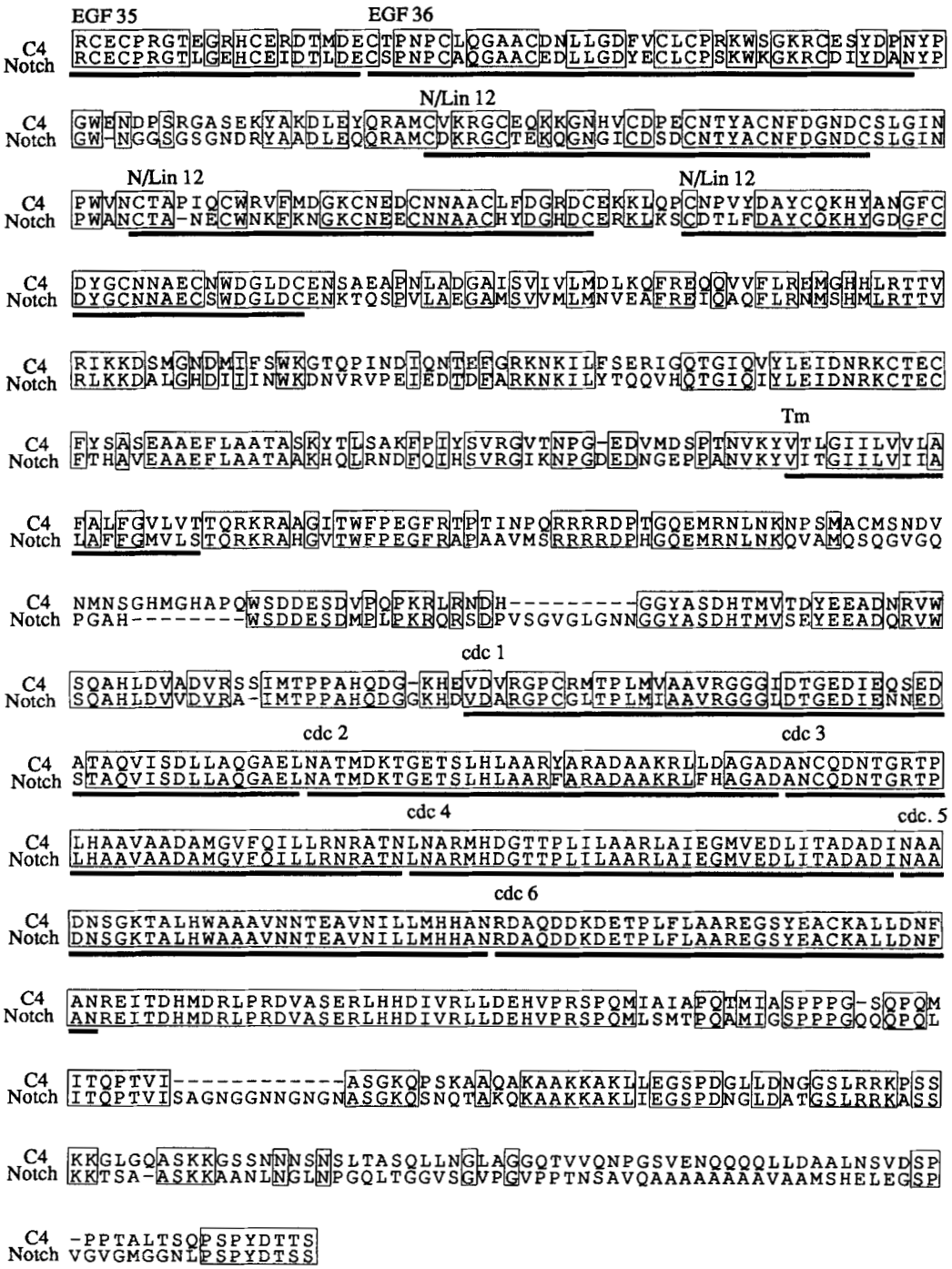

FIGURE 2.—Amino acid sequence based on the inferred translation product of the single long open reading frame in the *L. cuprina* clone, C4. The C4 sequence is aligned to the amino acid sequence of the NOTCH protein derived from exon E of the *D. melanogaster* gene. Conserved amino acids are boxed. Known NOTCH functional domains are underlined and identified with a label above the first amino acid.

particular alleles. The only completely penetrant phenotype observed in both "weak" and "strong" mutant alleles of *Scl* is the thickening of wing vein junctions, most obvious at the junction of the i-m crossvein and M_{1+2} vein (Figure 6).

Mutations in *Scl* also have significant effects on head and thoracic bristle (macrochaete) number. Expressivity of this phenotype is variable and incompletely penetrant. Generally, the wild-type pattern of bristles is altered in *Scl* mutants by a small, but significant, increase in the number of thoracic and head bristles (Figure 7). However, an increase in bristle number is not always the case as the loss of one or two bristles and their sockets can also occur less frequently (data not shown).

The large cytological deficiencies, *Df(3) w-Scl*^{2} and *Df(3) w-Scl*^{3}, display wing notching, wing vein thick-

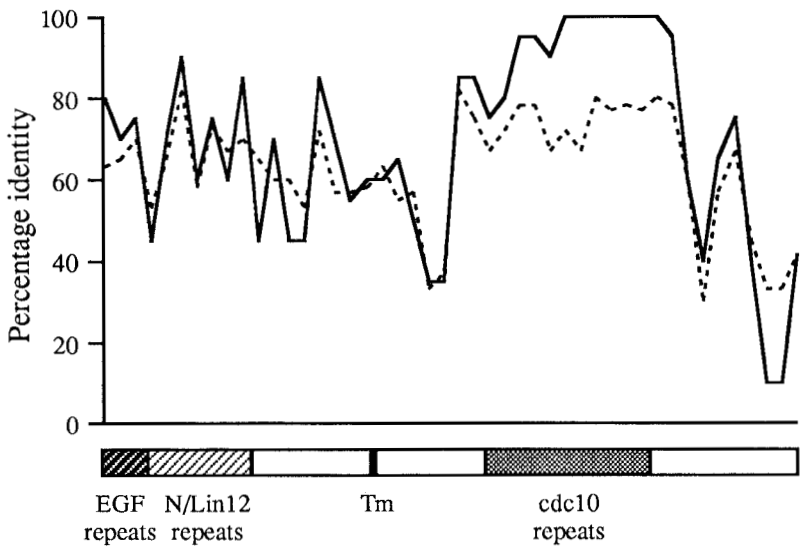

FIGURE 3.—Amino acid and nucleotide identity of C4 compared with the corresponding regions of NOTCH and *Notch*, respectively. The nucleotide identity is shown with a dashed line and the amino acid identity is shown with a solid line. Percentage identity is plotted on the *Y* axis. The NOTCH functional domains are identified on the *X* axis.

ening and increases in the number of macrochaete bristles similar to the remainder of the *Scl* mutants. Assuming that these deficiencies are true null alleles, which would be predicted given their cytology, then the similarity in phenotype suggests that the other *Scl* mutants are also loss-of-function alleles and represent nulls or hypomorphic mutations.

Recessive lethality and the embryonic lethal phenotype of *Scl* homozygotes: Recessive lethality was demonstrated through genetic crosses for each of the *Scl* alleles, Scl^1 *T(3:6)* Scl^2, Scl^3, Scl^4 and Scl^5, and the deficiencies, *Df(3) w-*Scl^2 and *Df(3) w-*Scl^3 (Figure 1; Table 1). In addition, each of these *Scl* mutations failed to complement the Scl^1 allele, lethality being associated with the *Scl/* Scl^1 heterozygotes in each case.

With the expectation that *Scl* homozygous mutant embryos may have a phenotype similar to that of *D. melanogaster Notch* mutant embryos (POULSON 1937; LEHMANN *et al.* 1983), *Scl/ Scl* embryos were examined for an excess of neural tissue and a lack of ventral and cephalic epidermis. Antibodies against HRP, which bind specifically to neural tissue in insects (JAN and JAN 1982; SNOW *et al.* 1987), were used to highlight the developing nervous system. The embryos were examined mid-way through development (7 ± 0.5 hr old at 27°) at an age corresponding approximately to stage 13 of *D. melanogaster* embryogenesis (CAMPOS-ORTEGA and HARTENSTEIN 1985). In the presumed *Scl* homozygotes, the increase in staining tissue reveals the nervous system hypertrophy that has occurred in these mutant embryos (Figure 8C). Each of the *Scl* alleles tested show a similar neurogenic phenotype to that shown in Figure 8C (data not shown) with the exception of Scl^5. The anti-HRP stained phenotype of Scl^5 homozygous embryos reveals some disorganization and increased staining limited to the anterior portion of the developing central nervous system (CNS) (Figure 8D). Thus Scl^5 appears to be a weak allele according to the neurogenic class system of LEHMANN *et al.* (1983) whereas the remainder of the *Scl* mutants conform with the "intermediate" and "extreme" classes.

Concomitant with an increase in the amount of neural tissue is a loss of epidermal tissue in the *Scl/ Scl* homozygous mutant embryos (Figure 9). The epidermis secretes the larval cuticle, therefore, where epidermis is absent, patches of cuticle will be missing (LOHS-SCHARDIN *et al.* 1979). In embryos homozygous for each of the *Scl* alleles, with the exception of homozygous Scl^5 embryos (see below), only the cuticle normally secreted by the dorsal epidermis is present (Figure 9B). In these embryos, the cuticle that remains includes posterior spiracles, which are connected to a portion of the tracheal stem and in most cases the remains of the pharynx (cephalopharyngeal skeleton) (Figure 9B). Once again, the absence of cephalic and ventral epidermis places these *Scl* alleles in the intermediate and extreme classes described by LEHMANN *et al.* (1983). In the case of the weak allele, Scl^5, homozygous embryos display both dorsal and ventral cuticle, which appear similar to wild type in pattern. However, the cuticle secreted by the cephalic region of the embryo is absent (Figure 9C). This is consistent with the anti-HRP staining phenotype observed for Scl^5 (Figure 8D) and compares closely with the weak cuticle phenotype described by LEHMANN *et al.* (1983).

Isolation of *Abruptex*-like alleles: Each of the previously existing *Scl* alleles can be classified as being equivalent to the hypomorphic *N* alleles of the *Notch* locus in *D. melanogaster*. To further extend the comparison of the *Scl* and *Notch* genes, a mutagenesis screen was carried out with the aim of isolating new alleles of *Scl* with phenotypes similar to the *Abruptex* (*Ax*) alleles of the *Notch* gene. Two new dominant mutations, which map to the region of *Scl*, were recovered from 18,940 progeny following EMS mutagenesis and visual screening. One mutation (Scl^{Ax1}) appears to correspond to

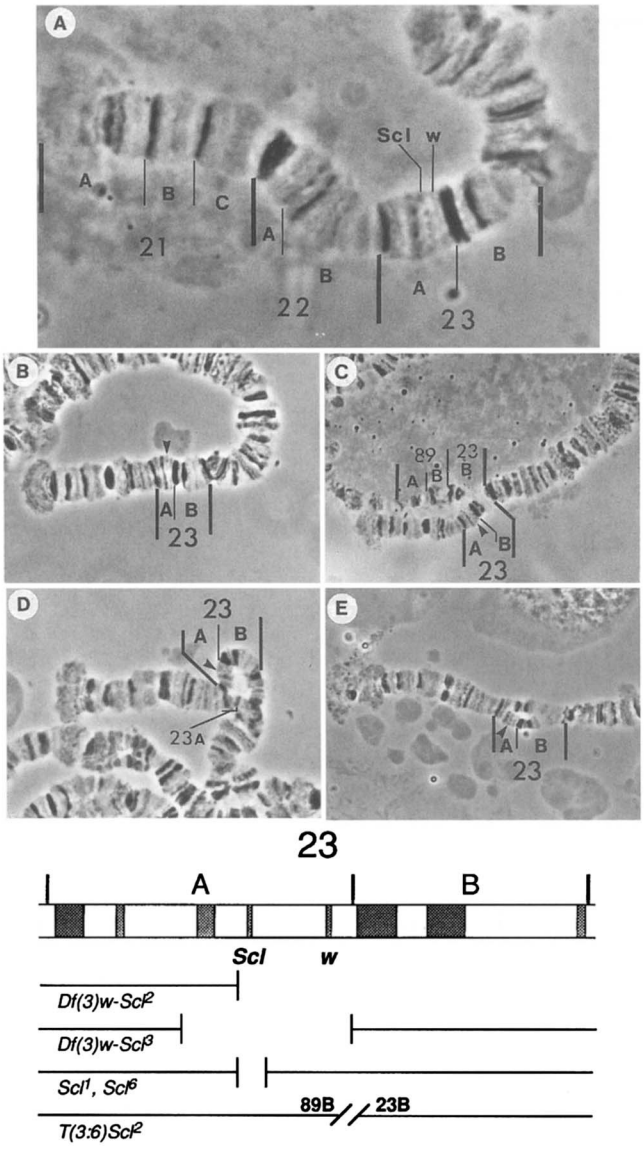

FIGURE 4.—Cytological localization of the *Scl* gene on pupal trichogen cell polytene chromosomes. (A) The *white* gene has been localized to the first minor band distal (left) of the 23A/B boundary on chromosome *III* (BEDO and HOWELLS 1987). The *Scl* locus is proposed to be the next minor band distal to the *white* band. (B–F) Polytene chromosomes from pupae heterozygous for the *Scl* allele indicated. Arrows indicate the putative *Scl* band on the wild-type portion of the polytene chromosomes. Distal is to the left. (B) The smallest cytologically visible *Scl* deletion, Scl^1, removes only the second band distal to the 23A/B boundary. The cytology of the Scl^6 mutation appears identical with that of the Scl^1 deletion (data not shown). (C) The breakpoint of the translocation in $T(3:6)\,Scl^2/+$ occurs at the 23A/B boundary, proximal to the *white* band. (D) The largest cytologically visible deletion that uncovers *Scl*, $Df(3)\,w\text{-}Scl^2$, breaks just distal to the predicted *Scl* band and in region 24A. (E) The deletion of $Df(3)\,w\text{-}Scl^3$ removes only the band distal to the predicted *Scl* band, the *Scl* band and the *white* band. (F) Summary of the cytologically visible mutations of *Scl*. With the exception of the mutation $T(3:6)\,Scl^2$, the cytological data would predict that *Scl* is in the region of the Scl^1 and Scl^6 deficiencies at the second band distal to the 23A/B boundary.

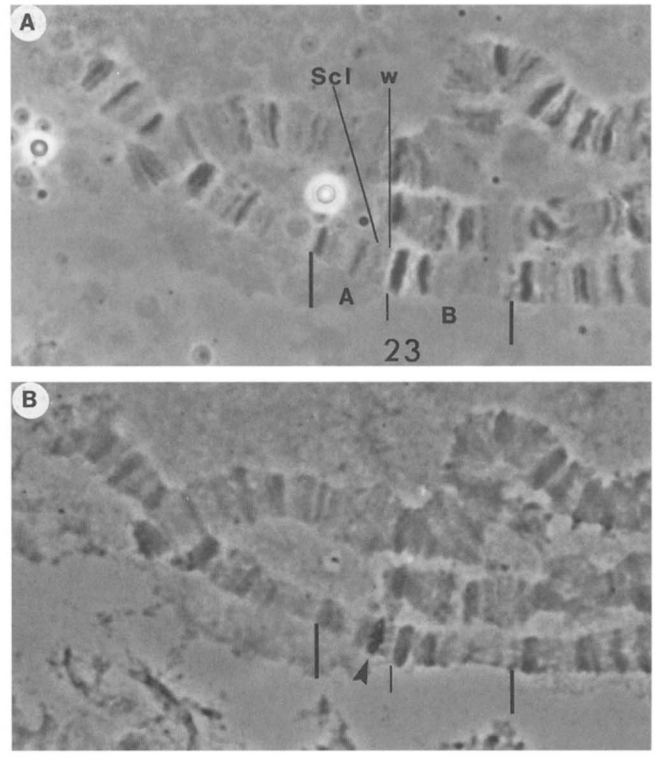

FIGURE 5.—Localization of the *Scl* gene by *in situ* hybridization to pupal trichogen cell polytene chromosomes. A and B are phase contrast micrographs of the same chromosome *III* preparation before and after hybridization. Distal is to the left. (A) Chromosome preparation before *in situ* hybridization. The proposed locations of the *Scl* and *white* (*w*) genes are marked. (B) The same chromosome preparation after *in situ* hybridization with a *Notch*-homologous *L. cuprina* genomic clone (λLcT1). The position of hybridization at the predicted *Scl* band is indicated with an arrowhead.

an allele of the recessive viable *Su*(*N*) class of *D. melanogaster Ax* alleles. The genetic behavior of the other (Scl^{Ax2}) is consistent with the recessive lethal (*l-Ax*) class of *Ax* alleles. Neither of these mutations has any visible cytological abnormalities when polytene chromosomes were examined (data not shown).

The mutant Scl^{Ax1} has partially dominant macrochaete loss from the thorax and head (Figure 10). Scl^{Ax1} homozygotes appear to have reduced viability; surviving homozygous Scl^{Ax1} adults show extensive macrochaete loss and nearly completely penetrant wing vein gaps (Figures 10 and 11). These phenotypes are consistent with the phenotypes of the recessive viable *Abruptex* alleles in *D. melanogaster* (LINDSLEY and ZIMM 1992).

Scl^{Ax1} behaves as a *Su*(*N*) *Abruptex* allele with respect to its complementation pattern with loss-of-function *Scl* alleles. The wing notching of these *Scl* alleles are partially or completely suppressed in heteroallelic combination with Scl^{Ax1} (Figure 11). The severity of the bristle and wing vein gapping phenotype of these Scl/Scl^{Ax1} heterozygotes tends to lie between those of the $Scl^{Ax1}/+$ heterozygotes and the Scl^{Ax1}/Scl^{Ax1} homozygotes.

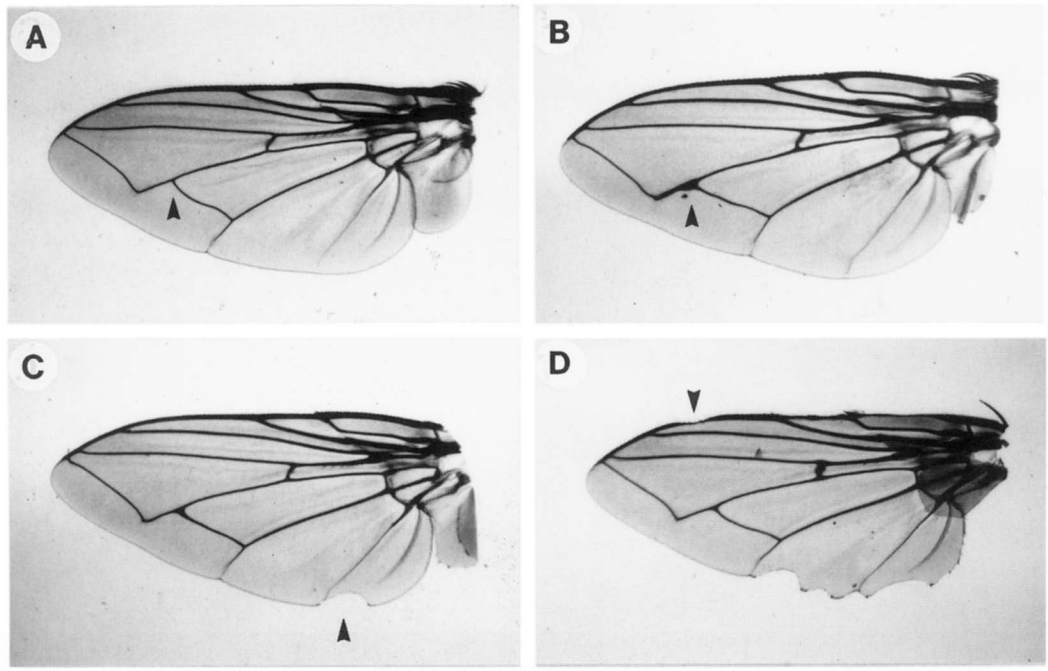

FIGURE 6.—Variation in expressivity of the *Scl* wing notching phenotype associated with loss-of-function alleles of *Scl*. The wing in A is wild type, all other wings are from *Scl*/+ heterozygotes. All wings are anterior to the right, the leading edge of the wing is at the top of each photograph and the trailing edge at the bottom. (A) Wild type. The arrowhead indicates the junction between the i-m crossvein and the M_{1+2} vein. (B) Weak *Scl* wing phenotype. The only fully penetrant wing phenotype associated with all *Scl* alleles is a thickening of the M_{1+2} vein and i-m crossvein junction (arrowhead). Note that the trailing margin of the wing is intact. (C) Mild *Scl* wing phenotype. The trailing margin of this wing has a single notch taken from it (arrowhead). The M_{1+2} vein and i-m crossvein junction is thickened. (D) A severe *Scl* phenotype. The M_{1+2} vein and i-m crossvein junction is thickened, there is disarray and loss of bristles on the anterior portion of the leading edge and the trailing margin is multiply notched.

The second *Ax*-like allele, Scl^{Ax2}, resembles a *l-Ax* allele. Scl^{Ax2} heterozygotes have dominant macrochaete loss from the thorax and head (Figure 10); at 20° they display wing vein gaps and wing margin notching, and at higher temperatures (25° and 27°) the wing margins are still notched but the vein gaps are replaced by thickening of wing vein junctions (Figure 11). Scl^{Ax2} is recessive lethal (see below). The combination of phenotypes for Scl^{Ax2} at 20° is similar to those observed for recessive lethal *D. melanogaster Ax* alleles at 17° and 25° although the wing margin notching is unusual (LINDSLEY and ZIMM 1992; DE CELIS and GARCIA-BELLIDO 1994). Significantly, all of the recessive lethal *D. melanogaster Ax* alleles show temperature sensitivity such that at 29° these alleles approach a *N*-like (loss-of-function) phenotype (PORTIN 1977; DE CELIS and GARCIA-BELLIDO 1994). The same appears to be true for Scl^{Ax2}; at 25° and 27°, heterozygotes no longer display vein gaps, instead wing vein junctions are thickened and the wing margins are notched (Figure 11).

Temperature shift experiments produce wing vein gaps (Figure 10) in developing Scl^{Ax2} flies transferred to 20° from 25° and 27° at the white prepupal stage, but not in flies shifted to 25° and 27° from 20° at the white prepupal stage. This pupal temperature-sensitive period differs from the findings of FOSTER (1973) with *D. melanogaster,* who found that temperature sensitivity for the vein gapping phenotype of Ax^{16172} was restricted to larval, not pupal, development.

Recessive lethality is associated with the Scl^{Ax2} allele (Table 1). This was determined using a cross similar to that shown in Figure 1 except the closer genetic marker w^m was used in the place of *ru*. In addition, the Scl^{Ax2} mutation was lethal in heteroallelic combination with Scl^1 at the normal rearing temperature of 27° (Table 1). Scl^{Ax2} homozygotes do not show an embryonic phenotype different from wild type either by anti-HRP staining or in cuticle pattern analysis (data not shown). Later developmental stages have not been examined closely, thus the nature and timing of the Scl^{Ax2} lethality is yet to be determined.

The wing notching phenotype of Scl^{Ax2} is suppressed by the Scl^{Ax1} mutation at 27°; Scl^{Ax1}/Scl^{Ax2} heterozygotes display completely suppressed or significantly reduced wing notching. At this temperature Scl^{Ax2} is behaving like the loss-of-function *Scl* alleles. In addition, the Scl^{Ax1}/Scl^{Ax2} flies have wing vein gaps that approach the severity of Scl^{Ax1}/Scl^{Ax1} homozygotes and an enhanced loss of macrochaete bristles from the thorax and head, similar to the Scl^{Ax1} homozygous phenotype. The viability of these flies appears to be greater than that of the Scl^{Ax1} homozygotes.

Interactions of alleles of *Scl* with the fitness/asymmetry *Modifier (M)* and *Rop-1*: *The effect of the Modifier on*

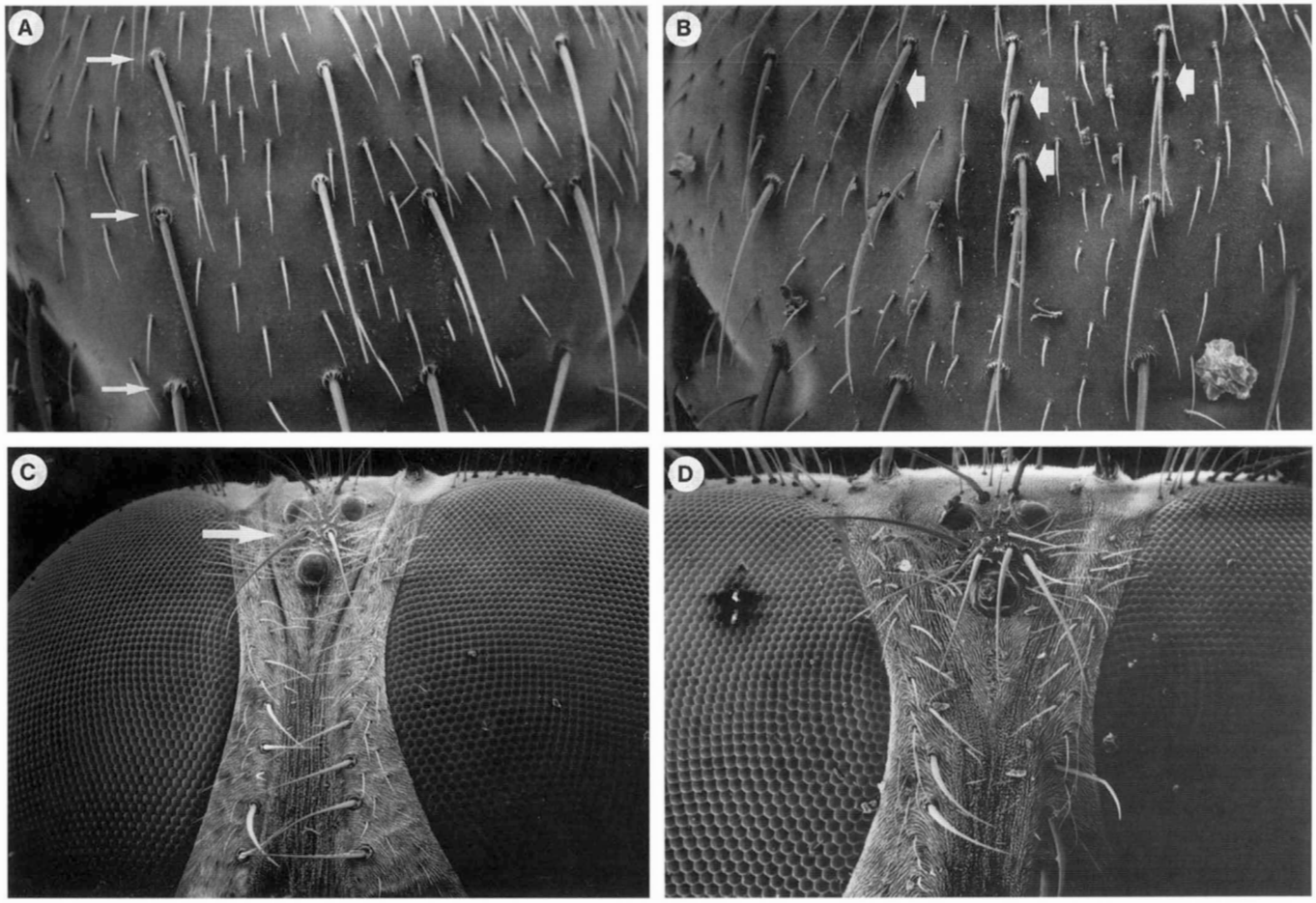

FIGURE 7.—The effect of loss-of-function mutations in *Scl* on the number of macrochaete bristles on the thorax and head. (A and B) Scanning electron micrographs showing a dorsal view of the dorsocentral and acrostichal bristles on the postsutural part of the mesonotum. Anterior is up and dorsal is down. (A) A wild-type distribution of macrochaete bristles. Three rows (thin arrows) of four bristles is the invariable arrangement of bristles on this part of the mesonotum in wild-type *L. cuprina*. (B) An example of the extra bristle phenotype in a *Scl*/+ heterozygote. The thick arrows point to four extra macrochaete bristles that are present in addition to the 12 normally found. (C and D) Scanning electron micrographs of an anterior view of the head. Dorsal is up. (C) Wild type. Bristles are arranged symmetrically on the frontal stripe and between the ocelli two bristles are highlighted (arrow). (D) *Scl*/+ heterozygote displaying additional bristles between the ocelli where there are now seven bristles instead of two. Although slightly disorganized there is no noticeable increase in the number of bristles in the frontal stripe.

Scalloped phenotype: Two *Scl* mutations (Scl^5 and Scl^{Ax1}) have incompletely penetrant phenotypes, both of which can be easily and precisely measured. Scl^5 heterozygotes display dominant incompletely penetrant notching of the trailing wing margin but dominant fully penetrant thickening of wing vein junctions. All heterozygous Scl^5 flies can be identified on the basis of the latter phenotype. Scl^{Ax1} has a dominant but incompletely penetrant missing bristle phenotype where bristles on the thorax and head can be absent. The incomplete penetrance of the bristle loss phenotype of Scl^{Ax1} and the wing notching of Scl^5 makes them useful for testing the hypothesized allelism of the fitness and asymmetry *Modifier* (*M*) of diazinon resistance with *Scl*. The effect of *M* was also tested on the Scl^4 allele, which has a fully penetrant wing notching phenotype.

The presence of *M* increased the proportion of flies expressing the Scl^5 wing notching phenotype in both susceptible (+/+) and resistant (*Rop-1*/+) backgrounds (Table 2). These results can be explained by increased penetrance of the wing notching phenotype in the presence of *M*. Viability differences between genotypes can be assessed because the completely penetrant thickened vein junction phenotype of Scl^5 allows identification of all *Scalloped* flies whether they express the wing notching phenotype or not. The proportion of total *Scalloped* flies to non-*Scalloped* flies was similar in the presence and absence of *M* (Table 2), thereby discounting differences in viability as a factor. We conclude that the presence of *M* increases the penetrance of the Scl^5 wing notching phenotype.

The effect of *M* on the phenotype of Scl^{Ax1} was more complex. Once again, in both susceptible and resistant backgrounds, the proportion of flies expressing the missing bristle phenotype was found to be greater in the crosses involving *M* (Table 2). These differences may

TABLE 1

Recessive lethality and noncomplementation of the loss-of-function *Scl* mutations and Scl^{Ax2}

Scl allele	Progeny of cross between Scl^{x} + *ru*/+ *w* + siblings			Progeny of cross between *In(3)*, Scl^{1} + *ru*/+ *w* + and *Scl* + *ru*/+ *w* +		
	No. of *Scl* ru^{+} progeny	No. of *w* progeny	No. of *Scl ru* progeny	No. of *Scl* ru^{+} progeny	No. of *w* progeny	No. of *Scl ru* progeny
Scl^{1}	319	291	17	178	161	0
$T(3:6)Scl^{2}$	317	106	24	157	111	0
Scl^{3}	128	97	8	259	147	0
Scl^{4}	237	173	8	123	86	0
Scl^{5}	197	98	19	184	121	0
$Df(3)w\text{-}Scl^{2}$	219	332	2	169	107	0
$Df(3)w\text{-}Scl^{3}$	65	47	2	235	186	0
Scl^{Ax2}	1022[a]	581	0[a]	138	68	0

[a] The closer genetic marker w^{m} was used to mark the Scl^{Ax2} chromosome instead of the *ru* mutation. w^{m}/w^{m}, w^{m}/w and w/w genotypes are distinguishable.

be due to an increase in the penetrance of the Scl^{Ax1} phenotype, as was the case for the Scl^{5} crosses. The possibility of an increase in the viability of the M/Scl^{Ax1} flies compared with $+/Scl^{Ax1}$ flies cannot be eliminated on the basis of these data because $Scl^{Ax1}/+$ flies not expressing a phenotype could not be distinguished from their +/+ siblings. However, an observation that favors the possibility of an increase in the penetrance of the Scl^{Ax1} phenotype in the presence of *M* is that in a susceptible background, a small proportion (44/733) of the M/Scl^{Ax1} heterozygotes displayed weak wing vein gapping, a phenotype previously only observed in Scl^{Ax1}/Scl heteroallelic combinations. This phenotype was not witnessed in the progeny of any of the other crosses involving

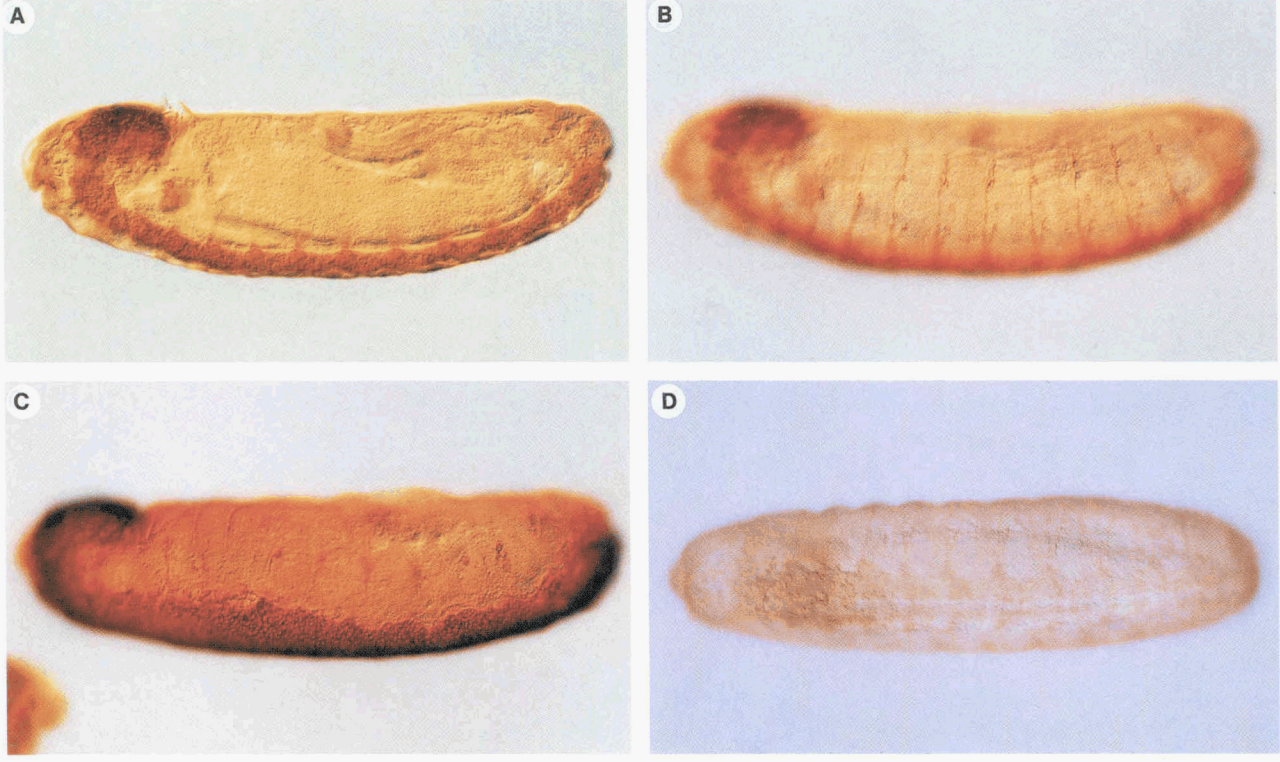

FIGURE 8.—Anti-HRP staining of the developing nervous system in wild-type (+/+) and *Scl*/*Scl* embryos. (A) Lateral view of a wild-type embryo. Focus is on the CNS, which runs from posterior to anterior on the ventral surface and expands in the cephalic region of the embryo. (B) A more lateral focus of the same embryo as in A such that the developing peripheral nervous system is visible. (C) Homozygous *Scl* embryo displaying a neurogenic phenotype. Staining cells cover the ventral and ventrolateral surface of the embryo from anterior to posterior. Dorsal structures appear unaffected. (D) Lateral view of a homozygous Scl^{5}/Scl^{5} embryo (weak hypomorph). Although slightly understained, the development of the nervous system appears wild type in this embryo except in the anterior cephalic region where disorganization and some excess neural staining is evident.

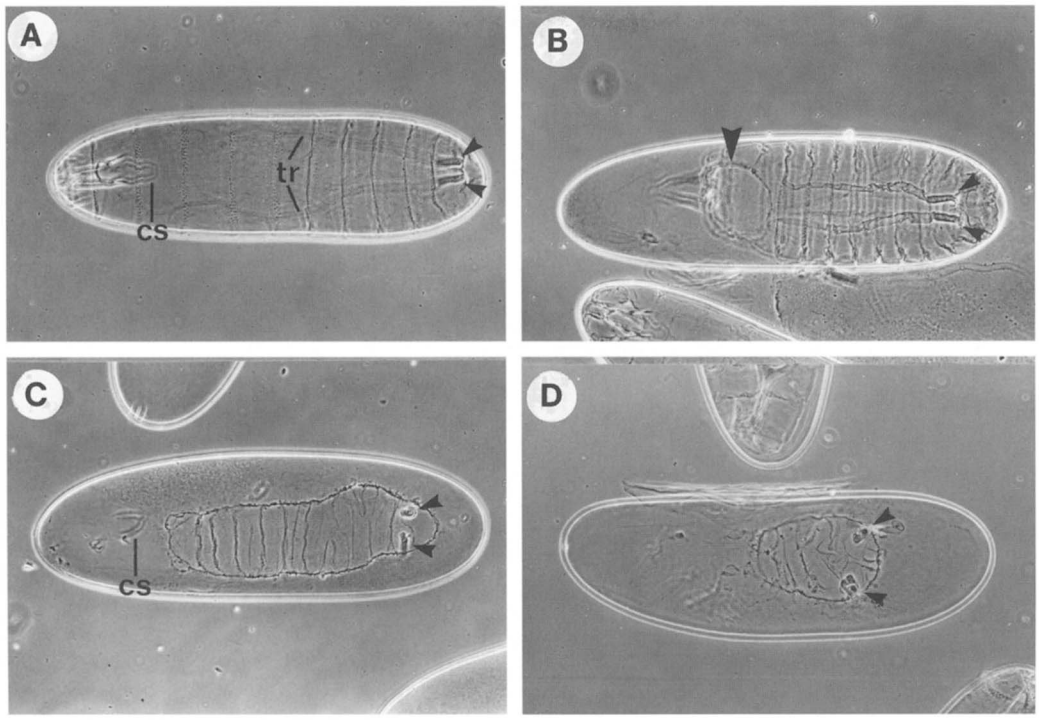

FIGURE 9.—Dorsal views of cuticle preparations of late stage wild-type (+/+) and *Scl*/*Scl* embryos. Anterior is to the left. (A) Wild-type embryo showing the segmental arrangement of denticle bands on the dorsal surface, the trachea (tr), which run the length of the embryo and the cephalopharyngeal skeleton (cs). (B) Scl^5/Scl^5 embryo (weak hypomorph). A complete loss of cuticle is observed in the cephalic region. A small region of the ventral cuticle is also missing resulting in a circular "hole" in the cuticle (large arrowhead). (C) Scl^1/Scl^1 embryo (strong hypomorph), only the dorsal cuticle and other structures associated with the dorsal cuticle such as the posterior spiracles (arrowheads), rudiments of the trachea and cephalopharyngeal skeleton are visible. The ventral and lateral epidermis is not present. (D) *Df(3) w-Scl*^{3}/*Df(3) w-Scl*^{3}. The most extreme phenotype observed, only a small patch of dorsal cuticle is present.

Scl^{Ax1}. While supporting the idea that an increase in penetrance is the likely cause for the changes in the frequency of missing bristles, this result also supports the hypothesis that *M* is allelic to *Scl* given that, in combination with Scl^{Ax1}, *M* generates a phenotype similar to that observed in *Scl*/Scl^{Ax1} heterozygotes.

The effect of *M* on the fully penetrant wing notching allele Scl^4 was only examined in a susceptible background. No differences were observed in the proportion of Scl^4 flies emerging in the presence and absence of *M* (Table 2), and there was no apparent difference in the expressivity of the wing notching phenotype (data not shown). Given the variation in notching normally associated with this and most of the loss-of-function mutations, any effect of *M* on expressivity would have to be extreme to be detected.

The Modifier affects the asymmetry of all Scl mutants: The asymmetry of each phenotypic class in the progeny of every cross was measured to allow a test of the behavior of *M*, the *Scl* alleles and the *Rop-1* allele in isolation and in various genotypic combinations. It would be predicted on the basis of previous experiments that the *Modifier* would reduce elevated asymmetry produced by the *Rop-1* allele and the *Scl* alleles (MCKENZIE and CLARKE 1988; MCKENZIE *et al.* 1990). Departures from this prediction may point to differences in the basis of the asymmetry.

Consistent with the findings of MCKENZIE *et al.* (1990), in a susceptible background the asymmetry of flies carrying each of the *Scl* alleles was elevated compared with their +/+ siblings (Figure 12). *M* affects this asymmetry in a partially dominant manner reducing the level of asymmetry of all three *Scl* mutants, but not to the basal level of non-*Scalloped* flies (Figure 12).

In a resistant background, *M* acts in the same partially dominant manner to reduce the asymmetry produced by the Scl^5 allele (Figure 12). The effect of *M* on the asymmetry of Scl^4 was not examined in a resistant background. The presence or absence of *M* appears to have no measurable effect on the level of asymmetry of Scl^{Ax1}/+ flies in a resistant background (Figure 12). This result is interesting because it suggests that a single copy of the resistance allele can affect the action of *M* on Scl^{Ax1} asymmetry levels. This difference may be attributable to the antimorphic nature of the Scl^{Ax1} mutation.

Scl and Rop-1 interact: Also measured in these crosses was a phenotypic interaction between the diazinon-resistance allele, *Rop-1*, and the Scl^{Ax1} and Scl^5 alleles. The penetrance of the Scl^5 wing notching phenotype is increased in a resistant background, the same effect as that of *M* but not as strong (Table 2). Together, *M* and the *Rop-1* allele have no greater effect on Scl^5 than does *M* alone.

The penetrance of the missing bristle phenotype of

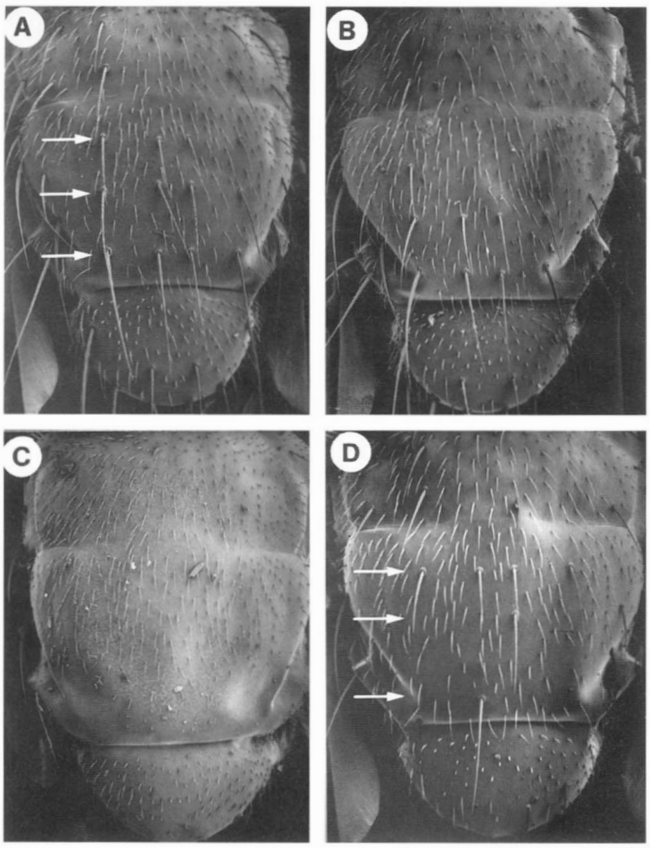

FIGURE 10.—Bristle phenotypes of the *Abruptex*-like alleles Scl^{Ax1} and Scl^{Ax2}. (A) Dorsal view of a wild-type thorax. Three rows of four bristles on the postsutural part of the mesonotum are highlighted (arrows). (B) $Scl^{Ax1}/+$. Two bristles are absent from the first row of four bristles. (C) Scl^{Ax1}/Scl^{Ax1}. All three rows of bristles are absent. The most posterior bristles on the scutellum remain. (D) $Scl^{Ax2}/+$. The bristle phenotype appears closer to that of Scl^{Ax1}/Scl^{Ax1} flies than $Scl^{Ax1}/+$ flies with the absence of many of the macrochaete bristles.

$Scl^{Ax1}/+$ flies in the presence of the *Rop-1* allele is reduced compared with that of $Scl^{Ax1}/+$ flies in a susceptible background (Table 2). This interaction is in the opposite direction to that produced by *M* in combination with Scl^{Ax1}. The presence of *M* in a resistant background (M/Scl^{Ax1}; $R/+$) increases the penetrance of the missing bristle phenotype but not to the level of $+/Scl^{Ax1}$;$+/+$ flies (Table 2). This comparison suggests that *M* and the *Rop-1* allele have opposing effects on the bristle loss phenotype with the *Rop-1* allele having a greater influence in suppressing the phenotype. In addition, the *Rop-1* allele has an opposite effect on the penetrance of the Scl^{5} wing notching phenotype compared with its effect on Scl^{Ax1}.

To summarize the effects of *M* and the *Rop-1* allele on the penetrance of Scl^{5} and Scl^{Ax1} phenotypes, *M* increases penetrance of phenotypes associated with both alleles in susceptible and resistant backgrounds. The *Rop-1* allele also increases the penetrance of the Scl^{5} wing notching phenotype in the absence of *M* but appears to have little effect in the presence of *M*. Conversely, the *Rop-1* allele decreases the penetrance of the Scl^{Ax1} missing bristle phenotype significantly in the absence of *M* and marginally in its presence.

DISCUSSION

***Scalloped wings* is the *L. cuprina Notch* homologue:** We have presented strong evidence supporting the hypothesis that the *Scalloped wings* gene of *L. cuprina* is the homologue of the *D. melanogaster Notch* gene. The *L. cuprina* subclone C4 shows close identity with exon E of *Notch* across the 2764 bp sequenced. Each of four characteristic *Notch* functional domains (EGF, Notch/Lin 12 repeats, transmembrane region and cdc10/ankyrin repeats) are present in the same order and spacing as in *Notch*. Given that *Notch* appears to exist as a single copy gene in insects, the C4 clone is likely to represent a portion of the *L. cuprina* homologue. That this gene is *Scl* is strongly supported by the *in situ* hybridization data, which localized the cloned sequence to the first band distal to the *white* band in region 23A on chromosome *3*, the cytological position of the *Scl* gene.

The close identity of *Scl* and *Notch* at the molecular level translates to a functional similarity revealed by the genetic and phenotypic analyses. Both genes play a role during embryogenesis in determining the neural/epidermal fate of cells in the neuroectoderm as the analysis of the homozygous lethal embryonic phenotypes indicates. Loss-of-function mutations in each gene result in near identical dominant adult phenotypes, evidence that the parallel functions of the two genes continues throughout development. Other *Notch*-related developmental processes were not examined although it would be predicted that *Scl* function is likely to mirror the pleiotropy of *Notch*. Therefore we would expect that *Scl* will also have a role in oogenesis (RUOHOLA *et al.* 1991; XU *et al.* 1992), spermatogenesis (XU *et al.* 1992), embryonic mesoderm formation (CORBIN *et al.* 1991; BATE *et al.* 1993) and eye development (SHELLENBARGER and MOHLER 1975; CAGAN and READY 1989; FORTINI *et al.* 1993), all developmental processes in which *Notch* has been shown to act in *D. melanogaster*.

The isolation of two *L. cuprina Abruptex*-like mutations (Scl^{Ax1} and Scl^{Ax2}) gives further support to a functional *Scl/Notch* homology. The observed phenotypes of Scl^{Ax1} define it as an *Ax*-like allele although the recessive nature of the wing vein gap phenotype is uncommon although not inconsistent with the *Ax* class of alleles of *Notch*. The Scl^{Ax2} allele appears to show a hybrid phenotype of both hypomorphic *N* alleles and hypermorphic *Ax* alleles. This is a characteristic of the recessive lethal *Ax* alleles of *Notch* (DE CELIS and GARCIA-BELLIDO 1994). At lower temperatures the *D. melanogaster l-Ax* alleles display the vein gaps and missing bristles common to all *Ax* alleles. At 29°, these *l-Ax* alleles resemble the loss-of-function *N* alleles and behave as such in genetic combinations with other *Notch* alleles (PORTIN

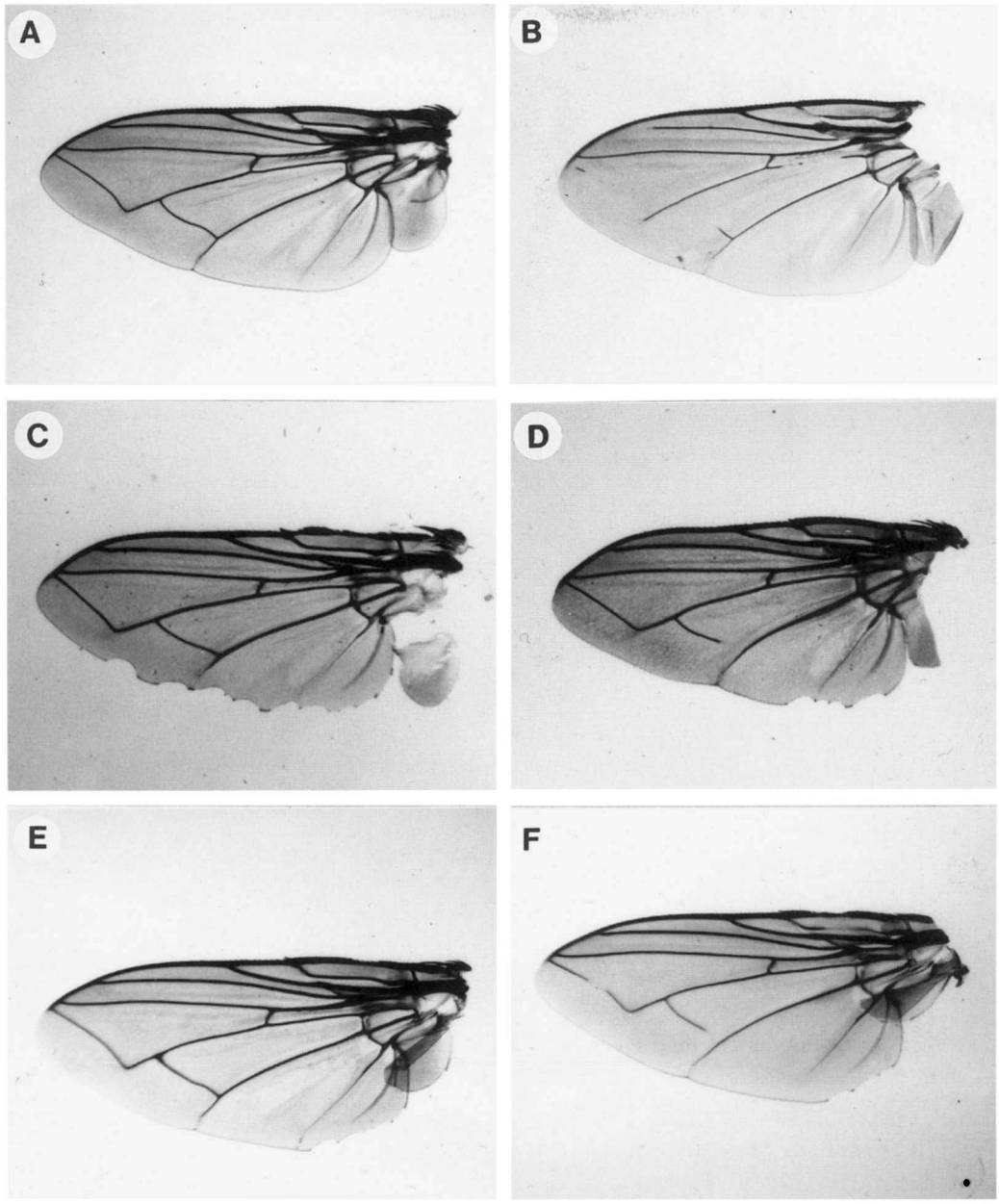

FIGURE 11.—Wing phenotype of the *Abruptex* alleles Scl^{Ax1} and Scl^{Ax2} and the interaction of Scl^{Ax1} with loss-of-function *Scl* alleles. (A) Wild type. (B) Scl^{Ax1}/Scl^{Ax1}. Vein gaps occur in the M_1 and i-m cross-vein, the anterior part of the M_{1+2} vein and the posterior part of the R_{4+5} vein. (C) $Scl^{1}/+$. A loss-of-function allele showing characteristic notching of the trailing wing margin and thickening of wing vein junctions. (D) Scl^{1}/Scl^{Ax1}. Wing notching is suppressed and the Scl^{Ax1}/Scl^{Ax1} vein gap phenotype is evident although not as severe as Scl^{Ax1}/Scl^{Ax1} homozygotes. (E) $Scl^{Ax2}/+$. At 25° or 27°, the trailing wing margin is notched and the M_{1+2} vein and i-m cross-vein junction is thickened, identical to the loss-of-function *Scl* alleles. (F) $Scl^{Ax2}/+$. At 20°, the notching of the trailing margin remains, the vein junction thickening is reduced or absent but most significant are the presence of gaps in the M_1 vein and the i-m cross-vein.

1977; DE CELIS and GARCIA-BELLIDO 1994). At 20°, Scl^{Ax2} displays a hypermorphic vein gap phenotype, at 25° and 27° there is the hypomorphic phenotype of thickened wing vein junctions. At all temperatures both hypomorphic wing margin notching and hypermorphic bristle loss occur, suggesting that *Scl* function may be affected to varying degrees in different developing tissues in this mutant. The wing margins appear to be the most sensitive to altered *Scl* function because they always display the loss-of-function phenotype.

Significantly, all of the phenotypes associated with alleles of *Scl,* both dominant and recessive, can be associated with alleles of *Notch,* suggesting that indeed there is functional homology between *Scl* and *Notch.* This implies that *Scl* and *Notch* function in the formation of the same tissues and a change or loss of function in those tissues is capable of producing similar developmental defects. This functional homology is important because of the postulated allelism of the *Modifier* of fitness and asymmetry (*M*) of diazinon resistance with the *Scl* gene.

TABLE 2

The penetrance of *Scl* phenotypes in the presence and absence of *M* and *Rop-1*

Genotype of *Scl* parent	Genotype of non-*Scl* parent[c]			
	$\frac{+\ +}{+\ ,+}$	$\frac{M+}{M,+}$	$\frac{+\ Rop\text{-}1}{+\ ,Rop\text{-}1}$	$\frac{M\ Rop\text{-}1}{M,Rop\text{-}1}$
$\frac{Scl^5\ +^a}{+\ ,+}$	259/538 (0.48)	581/772 (0.75)	336/561 (0.60)	244/328 (0.74)
$\frac{Scl^{Ax1}\ +^b}{+\ ,+}$	853/1224 (0.70)	733/820 (0.89)	292/689 (0.42)	249/433 (0.58)
$\frac{Scl^4\ +^b}{+\ ,+}$	410/435 (0.94)	351/350 (1.00)	—	—

[a] The penetrance values for Scl^5 represent the number of Scl^5 heterozygotes expressing a wing notching phenotype over the total number of Scl^5 heterozygotes.

[b] The penetrance values for Scl^{Ax1} and Scl^4 represent the number of flies expressing the *Scl* phenotype (missing bristles for Scl^{Ax1} and wing notching for Scl^4) over the number of flies wild type for these features.

[c] This genetic nomenclature assumes that *M* is an allele of *Scl*.

Data from the comparison between *Notch* and *Scl* are important in providing a function for *M* as an allele of *Scl*. If allelism can be demonstrated and the developmental functions of *Notch* and *Scl* are the same, then the wealth of *Notch* literature can be used to suggest a mechanism for the fitness and asymmetry modification.

The *Modifier*, an allele of *Scalloped wings*?: The effect of *M* on the adult phenotype of two *Scl* alleles is consistent with *M* being an allele of the *Scl* gene. The incomplete penetrance of the wing notching phenotype in Scl^5 and the bristle loss phenotype of Scl^{Ax1} were utilized as a sensitive screen to measure an effect, if any, of *M*. The Scl^5 mutation was particularly useful because the wing vein thickening phenotype is fully penetrant so all flies carrying the Scl^5 allele could be identified and classed as having or not having notched wings, giving an accurate measure of the increase in penetrance produced by *M*. Crosses involving the Scl^{Ax1} mutation were less definitive in this regard as any differences observed might have been due to viability differences between genotypes. Evidence would suggest, however, that the observed differences are due to an increase in penetrance of the missing bristle phenotype. The assumption has been made in further discussion that differences in the proportion of flies missing or not missing bristles are due to changes in penetrance.

The significant increase in penetrance observed with both Scl^{Ax1} and Scl^5 when in the presence of *M* can be interpreted as a noncomplementing interaction between alleles of the same gene. The alternative is that the *Modifier* gene happens to map close to *Scl* and is involved in similar processes during adult development. The presence of wing vein gaps in some M/Scl^{Ax1} flies parallels the phenotype observed in heteroallelic combinations of Scl^{Ax1} and other alleles of *Scl*. This is perhaps the strongest evidence for the allelism hypothesis. That only a small proportion of M/Scl^{Ax1} flies have this phenotype can be explained by the weakness of the modifier phenotype. When homozygous and in isolation, *M* has no detectable phenotype. *M* does not appear to affect the fitness of susceptible flies (McKENZIE

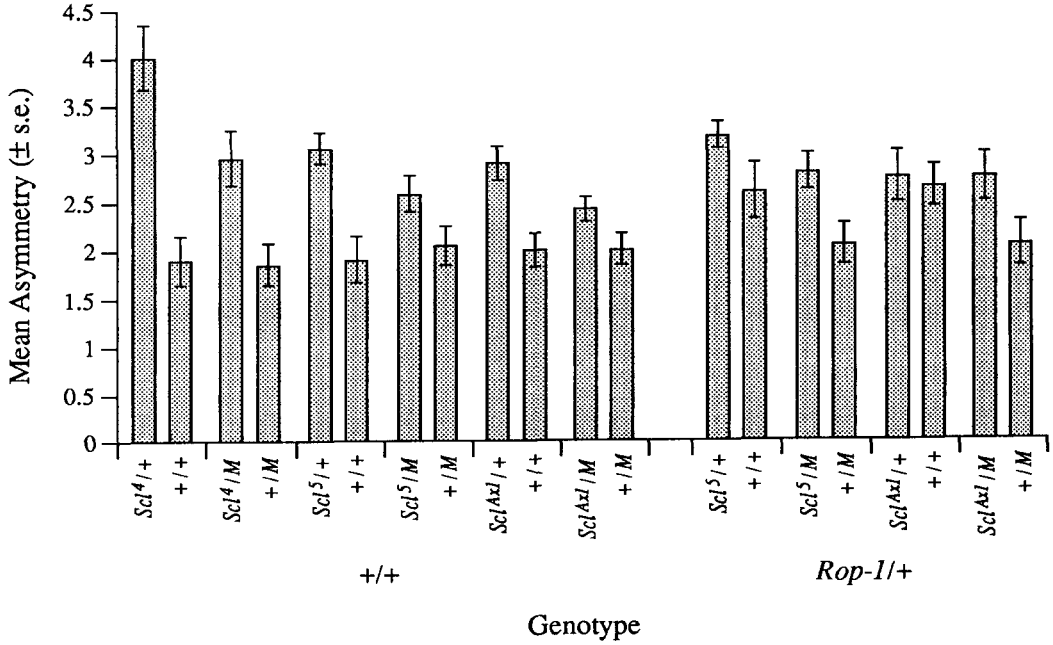

FIGURE 12.—Mean asymmetry (±SE) of flies heterozygous for an allele of *Scl* (Scl^4, Scl^5 or Scl^{Ax1}) and their non-*Scalloped* siblings (+) in the presence and absence of *M* in either susceptible (+/+) or resistant (*Rop-1*/+) backgrounds. The genetic notation assumes allelism between *M* and *Scl*.

and GAME 1987). This suggests that an effect, if any, on visible mutations could be subtle. This is confirmed by the lack of detectable phenotypic changes when *M* is in heterozygous combination with Scl^4.

MCKENZIE *et al.* (1990) present evidence that *M* increases the viability of Scl^1 flies and decreases their developmental time, an indication of an increase in fitness. In those experiments, flies were assigned the Scl^1 genotype if they possessed notched wings. Flies were not assessed on the presence or absence of thickened vein junctions. As the Scl^1 phenotype is incompletely penetrant (MADDERN *et al.* 1986), an increase in wing notching penetrance of the M/Scl^1 flies could also account for the apparent increase in viability. These experiments need to be repeated using the thickening of wing veins as the marker for Scl^1.

The interaction between the *Rop-1* gene and *Scl*, seen as changes in the Scl^5 and Scl^{Ax1} adult phenotypes, was previously unknown. This relationship, if true, can also be taken as evidence for the allelism between *Scl* and *M* on the basis of mutual interactions. *M* was identified because of its effect on *Rop-1* phenotype (MCKENZIE and GAME 1987; MCKENZIE and CLARKE 1988). The interaction between *Scl* and *Rop-1* described here is most simply explained if *M* and *Scl* are the same gene such that there is an interaction between two genes instead of three.

Interaction between the *Scl* and *Rop-1* products—a hypothesis: The *Rop-1* allele reduced the penetrance of the bristle loss phenotype of Scl^{Ax1} and increased the penetrance of the Scl^5 wing notching. Observing such an interaction between *Rop-1* and the Scl^5 and Scl^{Ax1} alleles at the phenotypic level was surprising, but it is an interaction that might be predicted if *M* is an allele of *Scl*. These data suggest the possibility of a role for *Rop-1* in bristle and wing development. If this is true, then it creates a scenario for a direct interaction between *M*, as an allele of *Scl*, and *Rop-1* in the modification of the developmental stability of resistant genotypes.

Although the wild-type function of the *Rop-1* gene product is not well understood, there is evidence to further support a role in bristle and wing development. *Rop-1* and *Scl* are coexpressed in the eye and wing imaginal discs of late third instar larvae and in the wing discs during the first 3 hr of pupal development (R. BURKE and P. BATTERHAM, unpublished data). This time of expression overlaps with the temperature-sensitive period for increased bristle asymmetry determined for a temperature-sensitive *Rop-1* mutation (J. MCKENZIE and P. BATTERHAM, unpublished data).

The *Rop-1* gene encodes the carboxylesterase E3 (HUGHES and RAFTOS 1985; PARKER *et al.* 1991), which shows significant similarity with acetylcholinesterase (AChE) (R. NEWCOMB, R. RUSSELL and J. OAKESHOTT, personal communication). Both these proteins have similarity with the *D. melanogaster* adhesion molecules neurotactin and glutactin (OLSON *et al.* 1990; HORTSCH and GOODMAN 1991). Glutactin is a basement membrane protein expressed throughout development (OLSON *et al.* 1990). Neurotactin has been characterized as a transmembrane protein expressed in the embryonic and larval nervous system and in the developing eye and wing imaginal discs where the protein is localized at points of cell contact (DE LA ESCALERA *et al.* 1990; HORTSCH *et al.* 1990). The similarity between neurotactin and AChE is such that if the extracellular domain of Neurotactin is replaced with the equivalent domain from AChE, then this chimaeric protein, when expressed in cultured Drosophila S2 cells, is capable of producing heterophillic aggregation between cells (M. PIOVANT, personal communication). It seems possible then, that in addition to their characterized enzymatic functions as esterases, the AChE and E3 proteins may also play a role in mediating cell adhesion (BALASUBRAMANIAN and BHANUMATHY 1993).

To relate an adhesion mechanism to the proposed interaction between *Scl* and *Rop-1*, it is not necessary that the Scl and E3 proteins be interacting directly, instead a situation can be hypothesized where the carboxylesterase E3 might promote adhesion between cells that are undergoing a developmental fate decision requiring the Scl protein. In the absence of molecular data our working hypothesis proposes that the *Rop-1* resistance mutation alters the adhesive properties of E3 in addition to changing the substrate specificity of the esterase activity, which produces diazinon resistance. This change in adhesion leads to developmental instability and increased asymmetry through an effect on the *Scl/Notch* signalling pathway. Clearly the influence on development is subtle given the only visible phenotype is bristle asymmetry. However, in these flies there is still E3 protein being produced by the resistance gene, albeit a product with an altered function. This hypothesis would predict an increased effect on bristle development in a *Rop-1* null fly.

M is proposed to be a compensatory mutation in the *Scl* gene such that developmental stability is restored and asymmetry is reduced. Identification of the *M* mutation should be informative with regard to predicting how that compensation takes place. A mutation in the extracellular domain of Scl might influence the interaction of Scl protein with its ligands or may simply increase its adhesiveness. A mutation of the intracellular domain might affect the transmission of a signal to cytoplasmic proteins, which would be predicted to bind to Scl as they do with Notch (DIEDERICH *et al.* 1994; ARTAVANIS-TSAKONAS *et al.* 1995).

The influence of the *Modifier* on asymmetry levels is difficult to explain given that it has a similar effect on all resistant mutations of *Rop-1* and every *Scl* mutation with which it has been tested. Either all of the mutations in these two genes create similar defects that the *Modifier* can correct or the *Modifier* acts in a dominant and

independent fashion. The only exception to this is the lack of influence *M* has on the elevated asymmetry associated with Scl^{Ax1} in a resistant (*Rop-1*/+) background. The antimorphic phenotype of bristle loss and vein gapping associated with Scl^{Ax1} sets it apart from the remaining hypomorphic *Scl* mutations tested in combination with *M*. Why the asymmetry associated with Scl^{Ax1} is only reduced in a susceptible background may only be explained when the proposed interaction between Scl and E3 is better elucidated. It should be noted that *M* does not have a general influence on asymmetry; *M* does not reduce the elevated asymmetry levels associated with mutations in the dieldrin-resistance gene *Rdl* (CLARKE and MCKENZIE 1987; MCKENZIE *et al.* 1990), an insect GABA receptor-chloride channel (ffRENCH-CONSTANT *et al.* 1991; K. FREEBAIRN and P. BATTERHAM, unpublished data). It does, however, reduce the asymmetry associated with another organophosphorous insecticide resistance gene, *Rmal* (MCKENZIE and O'FARRELL 1993). Given that *Rop-1* and *Rmal* are members of a multigene family (R. NEWCOMB, R. RUSSELL and J. OAKESHOTT, personal communication), it is possible that the modifier specifically interacts with these esterases and related gene products.

The diazinon-resistance system, its effects on development and, as a consequence, fitness, and the role of the *Modifier* in limiting those effects offers a striking example of rapid molecular evolution in response to selection. The model we have proposed can explain why the incorporation of a new allele (*Rop-1*) into a coadapted genome leads to developmental perturbation, in this case by a direct role of the *Rop-1* gene in bristle and wing development, possibly through a role in cell adhesion. Further selection on the disrupted genome eliminated the detrimental effects of the new allele by mutation in an interacting gene, proposed to be *Scl*, which could compensate for the changes in the adhesive properties of the carboxylesterase E3 without affecting the altered esterase activity, which was absolutely required for viability in the face of strong selection by insecticide.

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