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Genetic and Cytogenetic Analysis of the 43A-E Region Containing the Segment Polarity Gene *costa* and the Cellular Polarity Genes *prickle* and *spiny-legs* in *Drosophila melanogaster*

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ABSTRACT

A cytogenetic analysis of the 43A-E region of chromosome *2* in *Drosophila melanogaster* is presented. Within this interval 27 complementation groups have been identified by extensive F_2 screens and ordered by deletion mapping. The region includes the cellular polarity genes *prickle* and *spiny-legs*, the segmentation genes *costa* and *torso*, the morphogenetic locus *sine oculis* and is bounded on its distal side by the eye-color gene *cinnabar*. In addition 19 novel lethal complementation groups and two semi-lethal complementation groups with morphogenetic escaper phenotypes are described.

THE chromosomal region 43A-E lies within the proximal region of the right arm of chromosome two in *Drosophila melanogaster*. Seven genetic complementation groups have previously been described within this region: *prickle* (*pk*), *spiny-legs* (*sple*), *l(2)pawn* (*l(2)pwn*), *costa* (*cos*), *sine oculis* (*so*), *torso* (*tor*) and *cinnabar* (*cn*). Mutations at all of these loci give morphogenetic defects with the exception of *cn*, which gives an eye-color phenotype. The objective of this study was to provide a fine-scale genetic analysis of the region surrounding *pk*, *sple* and *cos* as a basis for the molecular analysis of these genes. A number of new complementation groups and chromosomal rearrangements are described and used to construct a genetic map and cytological map of the 43A-E region (Figure 1).

Mutant alleles of *pk* and *sple* affect the polarity of bristles and hairs (GUBB and GARCÍA-BELLIDO 1982), whereas those of *l(2)pwn* affect bristle and hair morphology (GARCÍA-BELLIDO and DAPENA 1974). Embryos mutant for loss of function alleles of *cos* that are derived from a mutant female germ line display defects in the larval segments. A posterior part of the segmental denticle belt is missing and is replaced by a mirror-image duplication of the anterior part including the segment boundary (GRAU and SIMPSON 1987). Embryos derived from females mutant for *tor* lack the terminal acron and telson (SCHÜPBACH and WIESCHAUS 1986; SPRENGER, STEVENS and NÜSSLEIN-VOLHARD 1989; KLINGER *et al.* 1988; STRECKER *et al.* 1989). In addition, a *raf*-related sequence (*Draf2*) has been mapped to 43A2-5 (MARK *et al.* 1987). Prior to this study only a small number of rearrangements had been described in this region (GRAU and SIMPSON 1987).

MATERIALS AND METHODS

Stocks: The rearrangements used in this study are listed in Table 1. New mutations and aberrations recovered during this work will be described below and listed in Tables 1 and 2. Lethal loci are named according to their cytological position.

Crosses: Crosses were set up in vials cultured at 25°. Complementation crosses were between mutant chromosomes balanced over *In(2LR)O, Cy dp^{lvI} pr cn^{2}* (*CyO*). Mutations were classified as lethal if less than 1% of homozygotes survive to the adult stage and noncomplementary if transheterozygous flies expressed a mutant phenotype or no nonbalancer carrying progeny emerged among 150 or more progeny.

Mutagenesis: New mutations were induced in males with ethyl methanesulfonate (EMS) according to the protocol of LEWIS and BACHER (1968) or by X rays at a dose of 4,000 rads (100 kV, 10 mA for 5 min, 1.5-mm aluminum filter, Philips MG102 constant potential X-ray system, beryllium window).

Cytology: Chromosome aberrations were analyzed cytologically with temporary propionic-orcein-carmine squash preparations of larval salivary gland chromosomes. The aberrations were interpreted with the aid of the revised polytene chromosome maps of BRIDGES (see LEFEVRE 1976).

RESULTS

To characterize the *pk-cos* region, the first step was to recover a number of chromosome aberrations, in particular deletions, that could be used for the genetic mapping of lethal and visible mutations. The most productive of these screens was the reversion of *Droplet*, a gain-of-function mutation with a very clear dominant visible phenotype.

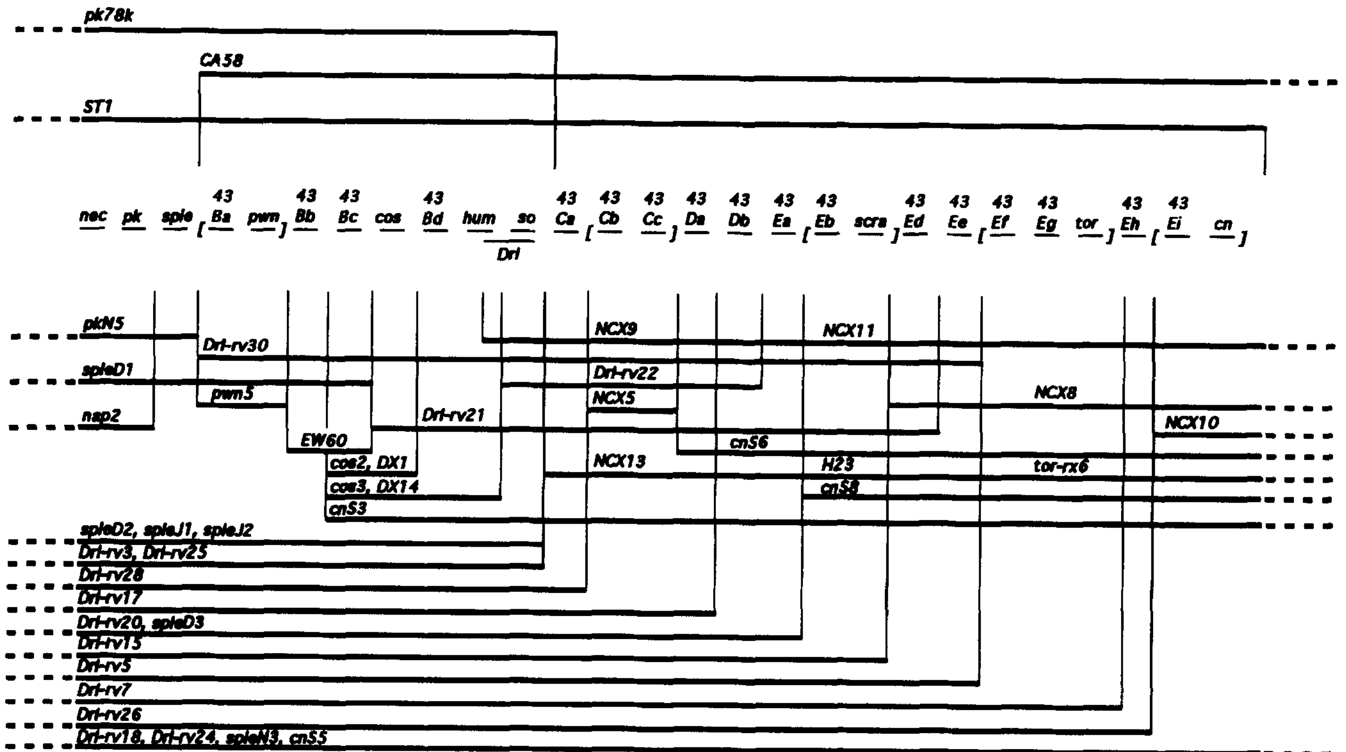

FIGURE 1.—A complementation map of the loci in the 43A-E region relative to each other and to chromosomal deficiencies. The order of loci and extents of deletions is shown, with the deletions used in the main screens drawn at the top of the figure, above the genetic loci. Loci that have not been ordered with respect to each other, either by a defining deletion end-point, or by recombination, are enclosed in square brackets. The *Df(2R)NCX9*, *Df(2R)NCX11* and *Drl* lesions are indicated as partially overlapping the *hum* locus as they fail to complement the sterility of female sterile *hum* alleles.

Reversion of *Droplet*: The dominant mutation *Droplet* (*Drl*) was induced by EMS and mapped to chromosome *2* to the left of *Tufted* (2-53.6 in region 37A3-6) by CRAYMER (1984). *Drl* causes an extreme reduction in the size of the eye when heterozygous with a wild-type chromosome. The first three X-ray induced revertants of *Drl* were recovered in a screen of 6,678 chromosomes in an experiment that was unrelated to this study. Of these revertants, two were found to be translocations each with one breakpoint in region 43B ($T(2;3)Drl^{rv1}$ and $T(2;3)Drl^{rv2}$, Table 1). This suggested that the original mapping of *Drl* might be incorrect and that the mutation could be related to *so* (2-57.1), which had been mapped to the 42E-43C interval (YANNOPOULOS *et al.* 1981). It was this experiment that suggested the use of *Droplet* reversion for the recovery of deletions in region 43.

Among approximately 100,000 non-Cy progeny of X-irradiated *Drl/CyO* males, 67 revertants of *Drl* were recovered and 29 stocks (Drl^{rv3} to Drl^{rv31}) were established. Sixteen of the 29 mutant second chromosomes were found to carry cytologically visible aberrations or were identified as chromosomal deletions by their failure to complement several loci adjacent to *so* (Table 1 and Figure 1). In view of our major objective, the recovery of deletions in region 43, we have not excluded the possibility that some of the chromosomally nonaberrant reversions result from dominant second-site suppressors of *Droplet*. In addition, four deletions including the *cn* locus were recovered among the *CyO* progeny in this screen (*Df(2R)cnS3*, *Df(2R)cnS5*, *Df(2R)cnS6* and *Df(2R)cnS8*, Table 1).

F_2 lethal screens: Four F_2 screens were performed to recover lethal mutations in the 43A to 43E region. In the first two screens, 3,400 EMS-treated bw^D chromosomes and 1,200 X-irradiated *cn bw sp* chromosomes were tested for visible and lethal mutations when heterozygous with the large deletion *Df(2R)ST1*. The first screen gave 39 EMS-induced lethal mutations and a single female sterile mutation. The second screen gave eight X-ray-induced lethals. To select mutations in region 43A-43E stocks of these mutations were crossed to *Df(2R)pk-78k* and *Df(2R)CA58* (Figure 1) and mutations that were viable with both these deletions, but lethal with *Df(2R)ST1*, were discarded. These crosses allowed us to discard any lethal chromosomes that might carry mutations elsewhere on chromosome *2*. These screens gave 31 EMS and seven X-ray-induced lethal mutations within the 43A to 43E interval (Table 2).

A further two screens were designed to recover lethal mutations mapping proximal to *pk*. In these

TABLE 1

Chromosomal rearrangements used in this study

Rearrangement	Cytology	Origin	Rearrangement	Cytology	Origin
Df(2R)CA58	43A3;43F6	a	*Df(2R)NCX5, bw sp*	Not visible	
Df(2R)cn-S3 + In(2LR)O,	43B1.2;44B7-9		*Df(2R)NCX8,* bw^D	43E5;44A1.2	
Cy dp^{lvI} *pr* cn^-			*Df(2R)NCX9,* bw^D	43C3-7;43F2	
Df(2R)cn-S5 + In(2LR)O,	42E4-F1.2;43E15-18		*Df(2R)NCX10,* bw^D	43F1.2;44C4.5	
Cy dp^{lvI} *pr* cn^-			*Df(2R)NCX11,* bw^D	43C1.2;44C1.2	
Df(2R)cn-S6 + In(2LR)O,	43C3-7;43F2-8		*Df(2R)NCX13,* bw^D	43C3-7;44B2-9	
Cy dp^{lvI} *pr* cn^-			*Df(2R)pk-78k*	42E3;43C3	e
Df(2R)cn-S8 + In(2LR)O,	43E7-18;44B9		*Df(2R)pk-N5, b* ap^{N5} *cn*	In(2R)41B-C;42F2-43A	
Cy dp^{lvI} *pr* cn^-			*Df(2R)pk-N6*	No data	
Df(2R)cos2, cn bw sp	Not visible	b	*Df(2R)sple-D1, b cn*	43A1.2;43B2	
Df(2R)cos3, bw sp	43B1	b	*Df(2R)sple-D2*	43A2;43C2	
Df(2R)cos13-rv1	No data		*Df(2R)sple-D3*	42E3-7;43C3-7	
Df(2R)Drl-rv3, bw	42E1-4;43C3		*Df(2R)sple-J1*	43A1;43C3-7	
Df(2R)Drl-rv5	42E4.5;43D7		*Df(2R)sple-J2*	43A2;43B3	
Df(2R)Drl-rv7	42D1;43E6		*Df(2R)sple-N3, b cn*	42E3-5;43F3-8	
Df(2R)Drl-rv15, b	43A3;43E7		*Df(2R)ST1,* Adh^{n5} *pr*	42B4.5;43E15-18	f
Df(2R)Drl-rv17	42C2-7;43C7		*Df(2R)tor-rx6*	43B1.2;43E15-18	
Df(2R)Drl-rv18	42E3-7;44A3		*Dp(2;2)*Sco^{rv1L}*TE35A-*4^R*, pr*	43A3.4;44C3	g
Df(2R)Drl-rv20	No data		*Dp(2;2)*Sco^{rv1L}*TE35A-*14^R	42E7;44C3	g
Df(2R)Drl-rv21	43B3;43E6		*Dp(2;3)P32,* pk^{32}; bx^{34e}	42F;44B;41A;42F;89E	h
Df(2R)Drl-rv22	43Cl;43D3-7		*In(2R)DX4, l(2)43*Bc^3 *cn bw sp*	41;43B1.3	
Df(2R)Drl-rv24	No data		*In(2R)DX8, l(2)43*Bc^5 *cn bw sp*	41;43B1	
Df(2R)Drl-rv25	42A14;43C7		*In(2R)*nec^3 pk^3, bw^D	41;43A1.2	
Df(2R)Drl-rv26	41F3;43E6.7		*In(2R)*nec^5 pk^5*, cn bw sp*	41B-C;42F1.2-43A1	
Df(2R)Drl-rv28	42D1;43B3		*In(2R)*pk^{19}*, b cn*	43A1-3;57C13.14	
Df(2R)Drl-rv30	43B1.2;43E5-7		*In(2R)Drl-rv16*	41A-E;43C1-3	
Df(2R)DX1	Not visible		*T(2;3)Drl-rv1*	43B3;86E14-20	
Df(2R)DX14, cn bw sp	43B1;43B3		*T(2;3)Drl-rv2*	43B3-8;91A3-8	
Df(2R)EW60, bw^D	Not visible		*Tp(2;2)Drl-rv23, bw*	41A-C;42A14-16;43C1-3	
Df(2R)H23	43B1.2;43F1.2	c	*Tp(2;2)pk-*$sple^{22}$*, b cn*	41E-F;42F3;43A1	
Df(2R)pwn-5, bw^D	Not visible		*Tp(2;3)DX11, l(2)43*Bb^3 *cn bw sp*	41;43B1-3;80	
Df(2R)nap-2, dp^v	41F4-9;43A1.2	d	*Tp(2;3)pk-*$sple^{24}$*, b cn*	41;43A1.2;81	

All chromosomes in Table 1 were obtained in this study unless otherwise indicated:

[a] *Df(2R)CA58* was found in a laboratory stock of *Dp(2;3)P32* and separated from the duplication. The origin of the deletion is unclear, but is probably *Df(2R)P32.*

[b] GRAU and SIMPSON (1987).

[c] G. REUTER (personal data).

[d] B. GANETZKY (personal data).

[e] GUBB and GARCÍA-BELLIDO (1982).

[f] S. TSUBOTA, described in ASHBURNER *et al.* (1981).

[g] Constructed using CRAYMER's method (1981) from inversions obtained by GUBB *et al.* (1986).

[h] Duplication element of *Tp(2;3)P32* (LEWIS 1963).

Df(2R)cos13-rv1 and *Df(2R)pk-N6* were lost before cytological analysis.

Df(2R)cos13-rv1 failed to complement *pk* and *cos*; *Df(2R)pk-N6* failed to complement all mutant chromosomes tested in the *nec-cn* interval.

screens, chromosomes were tested for lethality with *Df(2R)pk-78k* and counter-screened with *Df(2R)ST1*, so as to eliminate any lethals allelic to extraneous mutations on the *Df(2R)pk-78k* chromosome. A total of 5,470 EMS and 2,359 X-ray-mutagenized *b cn* chromosomes were tested giving a further 42 EMS-induced and eight X-ray-induced lethal mutations (Table 2). These new mutations were mapped with the available region 43A-43E deletions. Surprisingly, since the proximal cytological limits of both *Df(2R)pk-78k* and *Df(2R)ST1* are well proximal to 43B, all these mutations map distal to 43B, in the interval between *pk* and *so*, and define no additional complementation groups.

In addition to lethal point mutations, 17 visible mutations and one female sterile mutation were recovered in these F_2 screens, as well as five chromosomal deletions, two inversions and one transposition (*Df(2R)pwn-5*, *Df(2R)NCX5*, *Df(2R)EW60*, *Df(2R)DX1*, *Df(2R)DX14*, *In(2R)DX4*, *In(2R)DX8* and *Tp(2;3)DX11*).

F_1 screens for visible mutations: Two screens were made for mutations that failed to complement the wing phenotype of a hypomorphic *cos* allele (see below); 8,812 EMS-treated and 27,310 X-irradiated bw^D chromosomes were scored heterozygous with the cos^{11} *cn bw sp* chromosome. One new *cos* allele, cos^{12}, was recovered from the EMS screen. All putative new *cos* alleles from the X-ray screen were sterile. The *cn* mutation carried on the cos^{11} *cn bw sp* chromosome

TABLE 2

Mutant alleles recovered in this study

Complementation group	Allele	Progenitor chromosome	Mutagen
nec	*1, 2, 4*	bw^D	EMS
	6, 7, 8, 9, 10, 11, 12, 13	*cn bw sp*	EMS
pk	*2*	*cn bw sp*	X-ray
pk-sple	*6, 7, 8, 9, 25*	bw^D	EMS
	10, 14, 18	*b cn*	X-ray
sple	*4*	*cn bw sp*	X-ray
	5	*cn bw sp*	EMS
	27	*b cn*	X-ray
l(2)43Ba	*1, 2*	bw^D	EMS
	3	*cn bw sp*	X-ray
	4, 5, 6, 7	*cn bw sp*	EMS
l(2)pwn	*2, 3, 4*	bw^D	EMS
	5, 6	*cn bw sp*	EMS
l(2)43Bb	*1, 2*	bw^D	EMS
	3, 4, 5	*cn bw sp*	EMS
l(2)43Bc	*1, 2*	bw^D	EMS
	3, 4, 5, 6	*cn bw sp*	X-ray
	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	*cn bw sp*	EMS
cos	*8, 9, 10, 12*	bw^D	EMS
	13	*b TE35A*	EMS
	11, 14, 15, 16	*cn bw sp*	EMS
l(2)43Bd	*1, 2*	bw^D	EMS
	3	*cn bw sp*	X-ray
	4, 5	*cn bw sp*	EMS
hum	*1, 2, 3*	bw^D	EMS
	4	*b pr* $l(2)cos^{V1}cn$ *bw*	EMS
	fs5	bw^D	EMS
	fs6	*b pr* $l(2)cos^{V1}cn$ *bw*	EMS
	fs7	*b pr* $l(2)cos^{V1}cn$ *bw*	EMS
	8	cn bw sp	EMS
so	*3, 4*	bw^D	EMS
	5	*b cn*	EMS
	$Drl^{rv,6,8,10,11,13,14,27,29,31}$	*Drl*	X-ray
l(2)43Ca	*1, 2*	bw^D	EMS
l(2)43Cb	*1*	*cn bw sp*	X-ray
l(2)43Cc	*1, 2, 3*	bw^D	EMS
	4	*cn bw sp*	X-ray
l(2)43Da	*1, 2*	bw^D	EMS
l(2)43Db	*1*	bw^D	EMS
l(2)43Ea	*1*	bw^D	EMS
l(2)43Eb	*1*	bw^D	EMS
scra	*7, 8*	*cn bw sp*	X-ray
	9, 10	bw^D	EMS
l(2)43Ed	*1*	bw^D	EMS
l(2)43Ee	*1*	bw^D	EMS
l(2)43Ef	*1*	bw^D	EMS
l(2)43Eg	*1*	bw^D	EMS
l(2)43Eh	*1*	bw^D	EMS
fs(2)43Ei	*1*	bw^D	EMS
cn	*EW31, EW32*	bw^D	EMS
	NCX12	bw^D	X-ray
	S10	*In(2LR)O, Cy* dp^{lvI} *pr* cn^2	X-ray

also allowed the recovery of five X-ray-induced deletions that include the *cn* locus, *Df(2R)NCX8*, *Df(2R)NCX9*, *Df(2R)NCX10*, *Df(2R)NCX11*, and *Df(2R)NCX13*, and one new *cn* allele, cn^{NCX12}.

A similar screen to revert the adult phenotype of cos^{13}/cos^{V1} gave a single deletion, *Df(2R)cos13-rv1*, among 2,000 X-irradiated chromosomes. (The adult phenotype of cos^{13}/cos^{V1} flies resembles that of fused and is described below.)

Ten chromosomal aberrations in the region were recovered in screens for new *prickle* and *sple* alleles (D. COULSON, N. CHAPMAN and J. REGAN, unpublished data). Six of these aberrations were recovered from 48,374 X-irradiated chromosomes, *Df(2R)sple-D1*, *Df(2R)sple-N3*, *Df(2R)pk-N5*, *Df(2R)pk-N6*, $Tp(2;2)pk\text{-}sple^{22}$ and $Tp(2;3)pk\text{-}sple^{24}$. The remaining four, *Df(2R)sple-D2*, *Df(2R)sple-D3*, $Df(2R)sple\text{-}J^1$ and *Df(2R)sple-J2* were recovered from 32,380 chromosomes, by failure to complement the $sple^1$ mutation following the imprecise excision of a *P* element in the *pk* locus. The *P* element was mobilized by crossing the pk^{15} chromosome (D. COULSON, unpublished data) to a *b pr* $sple^1$; $P[ry^+(\Delta 2\text{-}3)](99B)$ stock. The $P[ry^+(\Delta 2\text{-}3)]$ *(99B)* chromosome carries a *P*-element insertion that provides constitutive high levels of expression of the *P* element transposase (ROBERTSON *et al.* 1988). Progeny of this cross were scored for the sple phenotype.

In addition, a viable allele of *so* (so^5) was recovered heterozygous with *Df(2R)NCX9* among 3,000 EMS mutagenized chromosomes. Individual *b cn*/*Df(2R)NCX9* males from this cross were mated to *Df(2R)ST1* virgin females in order to recover lethal mutations proximal to the 43B region (J. ROOTE, G. JOHNSON and T. MORLEY, unpublished data).

From these screens and existing mutant strains, 150 visible and lethal mutations were available for study.

Maternal effect lethals: Mutations affecting female fertility were not screened for sytematically. However, one of the novel lethal chromosomes recovered in this study, *humilis*, displayed a maternal effect (see below for description of *humilis, hum*). An F_2 screen of 2,700 EMS-treated chromosomes for mutations affecting female fertility was undertaken to isolate additional alleles of *hum*. This screen also produced six lethal mutations, which are included in the data in Table 2.

Alleles of the maternal-effect gene *torso* (*tor*) were obtained from C. NÜSSLEIN-VOLHARD and localized precisely within the 43A-E region. The maternal effect lethal, *scraps* (*scra*), described by SCHÜPBACH and WIESCHAUS (1989), also maps within this region and four novel zygotic lethal alleles were recovered in this study.

Deletion mapping of the 43A-E region: Mutations were first crossed to the *Df(2R)pk-78k* and *Df(2R)CA58* deletions to assign them to one of three regions (see Figure 1). The mutations in each group

TABLE 3

Recombination distances between some mutants in the *43A-E* region

Maternal genotype	Non-Cy progeny	Total progeny	Map distance (%)
$l(2)43Ba^1\ bw^D/pr\ pk\ l(2)pwn\ cn\ bw\ sp$	$pk^+\ cn^+$ 5, $pk^+\ cn$ 0, $pk\ cn^+$ 0, $pk\ cn$ 0	23,027	0.043[a]
$l(2)43Ba^2\ bw^D/pr\ pk\ l(2)pwn\ cn\ bw\ sp$	$pk^+\ cn^+$ 4, $pk^+\ cn$ 0, $pk\ cn^+$ 0, $pk\ cn$ 0	18,857	0.042[a]
$hum^1\ cn\ bw\ sp/l(2)43Bd^2\ bw^D$	1 cn^+	9,029	0.022
$cos^5\ cn\ bw\ sp/hum^1\ bw^D$	1 cn^-	4,039	0.050
$cos^5\ cn\ bw\ sp/l(2)43Bd^1\ bw^D$	1 cn^-	4,721	0.040
$cos^5\ cn\ bw\ sp/so^3\ bw^D$	1 cn^-	11,192	0.018
$sple^1/so^1$	$sple^+\ so^+$ 4, $sple\ so$ 0	3,304	0.24

Females of the above genotypes were crossed to *Df(2R)ST1, pr* pk^- cn^-*/CyO* males and the progeny scored according to the phenotypes listed for each cross.

[a] The lack of recovery of three of the four possible classes of Cy^+ progeny from these crosses suggests that the *pr pk l(2)pwn cn bw sp* chromosome carries a lethal mutation on either side of l(2)*43Ba* mapping within *Df(2R)ST1*. If this were the case, all recombinants in the *l(2)43Ba* to *l(2)pwn* interval would retain at least one lethal mutation and not be recovered. The surviving pk^+ cn^+ flies from these crosses should carry gene conversions of the *l(2)43Ba* lesion on either the $l(2)43Ba^1$ or $l(2)43Ba^2$ chromosomes.

were then crossed *inter se* and to the other deletions recovered in this study. Twenty-seven complementation groups were identified and ordered relative to each other by deletion mapping and recombination (Table 2).

This allows 16 of the complementation groups to be assigned a precise linear order with respect to adjacent loci (Figure 1). The remaining 11 loci occur in four pairs and a single group of three that are not ordered with respect to each other. The proximal extent of this region overlaps the distal boundary of the *Df(2R)nap-2* deletion recovered by B. GANETZKY (Table 1).

Exchange mapping: Several mutations surrounding *cos* were ordered by recombination (Table 3), giving results consistent with the deletion mapping. The region within which the largest number of mutations was recovered, between the proximal breakpoint of *Df(2R)CA58* and the distal breakpoint of *Df(2R)pk-78k* (Figure 1), corresponds to a genetic distance of about 0.24 map units, estimated as the distance between the *sple* and *so* loci (Table 3). The overall genetic extent of the region studied, between the proximal and distal breakpoints of *Df(2R)ST1*, corresponds to about 2.2 map units, estimated as the distance between the *pk* (2-55.3) and *cn* (2-57.5) loci.

Description of mutant phenotypes

necrotic: Amorphic mutations of *necrotic* are either pharate adult lethal, or die within 24 hr of eclosion. The adult flies look relatively normal at eclosion but, after a few hours, develop brown spots principally at the bases of the legs and around the ocelli. These patches become more extensive and darker as the flies age. Thirteen alleles of this gene have been studied (Table 2). With two exceptions, the EMS-induced nec^3 and the X-ray induced nec^5, the *nec* mutations appear to be amorphic, giving the same phenotype whether homozygous or when heterozygous with each other or a deletion.

Complete deletions of *necrotic*, indeed of *necrotic*, *prickle* and *spiny-legs*, may survive as adults (Table 4). These have a strong necrotic phenotype, and soon die. They also have a prickle phenotype (*e.g.*, if the proximal deletion is *Df(2R)nap-2*) or a prickle-spiny-leg phenotype (if *Df(2R)pk-N5*). Heterozygotes between *Df(2R)nap-2* and *Df(2R)pk-78k* are viable (3.8%) despite being homozygously deleted for the 7–8 bands between 42E3 and 43A1.2–a remarkably long region. This accounts for our failure to recover any lethal mutations in this interval (see above, also J. ROOTE, M. ASHBURNER and D. SEGAL, unpublished data).

The hypomorphic alleles, nec^3 and nec^5, were both recovered on chromosomes carrying an inversion in the region and an associated *pk* mutation (Table 1). $In(2R)nec^3\ pk^3$ gives a slight nec phenotype when homozygous, whereas $In(2R)nec^5\ pk^5$ is nec^+ when homozygous. When heterozygous with a deletion, both the $In(2R)nec^3\ pk^3$ and $In(2R)nec^5\ pk^5$ chromosomes give fertile adults that survive for more than a week. These flies have swollen abdomens, reddish spots that darken with age and a variable pk phenotype ranging from wild-type to weak pk. The viabilities of these two alleles on eclosion, heterozygous with a deletion, are somewhat higher than those of the amorphic alleles (Table 5).

Both nec^3 and nec^5 almost complement the amorphic *nec* alleles, giving a phenotype showing dark spots near the ocelli at a low penetrance. This is surprising and suggests that the 11 amorphic *nec* mutations might retain enough residual nec^+ activity to complement the hypomorphic alleles.

prickle* and *spiny-legs: *pk* and *sple* mutations affect the polarity of bristles and other cuticular processes

TABLE 4

The survival of complete deletions for the *nec, pk, sple* region, as demonstrated in heterozygotes between *Df(2R)nap-2* or *Df(2R)pk-N5* and distally extending deletions

	Df(2R)nap-2[a]		*Df(2R)pk-N5*[b]	
Df(2R)sple-J1	15/211	7.1%	19/124	15.0%
Df(2R)sple-J2	65/402	16.0%	20/217	9.2%
Df(2R)sple-D1	17/393	4.3%	64/971	6.6%
Df(2R)pk-78k	22/586	3.8%	4/167	2.4%

[a] Survivors necrotic and prickle.
[b] Survivors necrotic, prickle and spiny legs.
Numbers of Cy$^+$ progeny, over total numbers of progeny, from crosses between *CyO* balanced chromosomes.

TABLE 5

Viabilities of adult flies heterozygous for *nec* alleles and *nec*$^-$ deletions at eclosion; all mutant chromosomes were balanced over *CyO* and the numbers of non-Cy flies, over the totals, are shown. All survivors were phenotypically necrotic.

nec^1	180/1382	13%
nec^2	127/1024	12%
nec^3	121/357	34%
nec^4	110/565	20%
nec^5	75/252	30%
nec^6	44/445	10%
nec^8	47/379	11%
nec^9	35/187	19%
nec^{11}	38/318	12%

(GUBB and GARCÍA-BELLIDO 1982). These alterations in polarity differ from the mirror-image pattern duplications caused by segment polarity mutations in that the topological polarity of cuticular processes is affected without other elements of the pattern being altered. In the tarsi of *sple* mutations, however, extra tarsal joints are formed as mirror-image duplications (HELD, DUARTE and DERAKHSHANIAN 1986). These tarsal duplications are analogous to the intersegmental duplications seen in *cos*.

All known alleles of *pk* and *sple* are homozygous viable. Of nine new alleles of *sple* and two recovered as *pk*, eight were mutant for both *pk* and *sple* (*pk-sple*^{6}, *pk-sple*^{7}, *pk-sple*^{8}, *pk-sple*^{9}*9*, *pk-sple*^{10}, *pk-sple*^{14}, *pk-sple*^{18} and *pk-sple*^{25}). Seven of these have a double-mutant phenotype indistinguishable from each other and that of *sple*^{78a}, described in GUBB and GARCÍA-BELLIDO (1982). Double-mutant flies have rough eyes and weak pk and sple phenotypes. The double-mutant allele, *pk-sple*^{25}, has the rough-eyes typical of other double-mutant alleles of *pk-sple*, but a much weaker pk phenotype, visible only in mounted wings and affecting the polarity of hairs in the region of the posterior cross-vein. Three novel amorphic alleles of *sple* that were not also mutant for *pk*, *sple*^{4}, *sple*^{5}*5* and *sple*^{27}, were recovered in this study (Table 2).

This tendency to recover double-mutant alleles from an EMS screen suggests that *pk* and *sple* may be related genetic functions as EMS causes preferentially single base substitutions (WILLIAMS and SHAW 1987). In classical genetic terms, however, *pk* and *sple* are not allelic, the phenotype of *pk/sple* flies being wild-type (GUBB and GARCÍA-BELLIDO 1982). This is true for the original allele of *sple*, and also for the *sple*^{4}, *sple*^{5} and *sple*^{27}*27* alleles, when heterozygous with *pk*^{1}*1* or other single-mutant alleles of *pk*. When heterozygous with deletions that include both *pk* and *sple*, such as *Df(2R)pk-N5*, the new single-mutant alleles of *sple* give the amorphic *sple* phenotype described for *sple*^{1}/*Df(2R)pk-78k* (GUBB and GARCÍA-BELLIDO 1982) and single-mutant alleles of *pk* give a polarity phenotype similar to *pk*^{1}/*Df(2R)pk-78k* or *Df(2R)nap-2/Df(2R)pk-78k*.

Flies homozygous for a double-mutant allele have a less extreme prickle phenotype than *pk*^{1}/*pk*^{1} and a very weak polarity pattern in the leg. The abdominal polarity and the tarsal duplication phenotypes, however, remain similar to that of *sple* single-mutant flies (D. COULSON and D. GUBB, unpublished data). This surprising result does not reflect the double-mutant alleles being hypomorphic. When heterozygous with deletions including both *pk* and *sple*, the double-mutant alleles give a phenotype that is indistinguishable from that given by homozygous double-mutant alleles or that given by the overlapping deletion in *Df(2R)pk-N5/Df(2R)sple-D1* flies.

SHARMA, CHITNIS and SHYNGLE (1985) reported an unusual *sple* mutant, *sple*^{bor}, isolated from an EMS screen. When homozygous this has a stunted leg phenotype, as well as one affecting cell polarity on the legs. The *sple* mutation on this chromosome can, however, be separated from a second site mutation responsible for the stunted legs. This second mutation is allelic to *dachsund* (*dac* = *l(2)36Ae* of ASHBURNER *et al.* 1990) mapping to 35F on the left arm of chromosome 2. Once separated from *dac*, the *sple*^{bor} chromosome retains an amorphic *sple* mutation (renamed *sple*^{3} by LINDSLEY and ZIMM 1992), giving a phenotype similar to other *sple* alleles. A further single mutant allele of *pk*, *pk*^{22}, was identified within *Dp(2;3)P32*. This duplication is *nec*$^+$ *pk*^{32} *sple*$^+$ *pwn*$^+$, the *pk* mutation presumably mapping to the 42F breakpoint within the transposed segment.

l(2)pawn. The original allele of *l(2)pwn* was recovered in a screen for cell-viable marker mutations (GARCÍA-BELLIDO and DAPENA 1974). Homozygous clones within heterozygous flies give truncated bristles with transparent tips and thin, transparent hairs. The mutation is lethal in homozygous flies during the zygotic stage (GARCÍA-BELLIDO and DAPENA 1973). In

uncrowded cultures, occasional escapers survive, these show thin bristles and hairs and have dark brown eyes with melanic patches in the corneal cuticle (GARCÍA-BELLIDO and DAPENA 1974). Five new alleles were recovered in the present study (Table 2); all are phenotypically similar to the original allele.

costa: *cos* is a member of the segment polarity class of genes (GRAU and SIMPSON 1987; SIMPSON and GRAU 1987). The gene exhibits a strong maternal effect. Homozygous embryos from heterozygous mothers are lethal but develop until the end of embryogenesis and look normal except for occasional perturbations of the polarity of the ventral denticle belts. When derived from homozygous germ-line clones, however, homozygous *cos* embryos show extensive morphogenetic abnormalities. The thoracic denticle belts are absent and the abdominal denticle belts are reduced in width (GRAU and SIMPSON 1987).

A series of wild-type iso-alleles (the *V* alleles) were described that fail to complement lethal alleles only when heterozygous with dominant mutations of the *Costal* (*Cos*) (2-68) locus. One of the new alleles, cos^{13}, is unusual in that it fails to complement the wild-type iso-alleles in a Cos^{+} background. Such cos^{13}/cos^{V} flies lack ocelli, ocellar and post-vertical bristles and wing veins L3 and L4 are partially fused and lie closer together. In addition, the scutellum is reduced in size, scutellar bristles are missing and the wings are held-out. These phenotypes are characteristic of *fused* (BUSSON *et al.* 1988).

cos^{13} was recovered in a screen for lethal mutations in the *Adh* region (M. ASHBURNER, unpublished data). It maps genetically and by reversion between *pk* and *cn* and is lethal when heterozygous with lethal *cos* mutations or deletions that include the *cos* locus. The revertant *Df(2R)cos13-rv1* failed to complement *pk* and *cos*. It was suspected to be a very large deletion but was lost soon after recovery. It had a Minute phenotype that was suppressed by the presence of *Dp(2;3)P32*. Heterozygous $cos^{13}/+$ flies are essentially wild-type but have occasional held out wings and lack some post-vertical and scutellar bristles. This dominant phenotype, together with the failure to complement iso-alleles (in cos^{V}/cos^{13} flies), suggests that cos^{13} may represent a gain of function mutation.

Seven new lethal alleles, cos^{8}, cos^{9}, cos^{10}, cos^{12}, cos^{14}, cos^{15} and cos^{16}, in addition to a hypomorphic allele, cos^{11}, were recovered in the present screens (Table 2). cos^{11} is homozygous viable and wild-type, but when heterozygous with a deletion gives flies bearing pattern duplications. These flies are fertile, unlike flies homozygous for a second hypomorphic allele, cos^{7} (GRAU and SIMPSON 1987).

The *fused*-like phenotype of cos^{13} may be significant as *fused* is also a segment polarity gene. Furthermore, there is a dominant interaction between *fu* and *cos* (B. LIMBOURG-BOUCHON and T. PRÉAT, personal data): $fu^{1}/+$: $cos^{5}/+$ females have reduced or absent ocelli and ocellar bristles. This ocellar phenotype is also shown by $fu^{s}/+$; $cos^{5}/+$, $Df(1)fu^{Z4}/+$; $cos^{5}/+$, $fu^{A}/+$; $cos^{5}/+$ females and fu^{1}/Y; $cos^{5}/+$ males. In fu^{A}/Y; $cos^{5}/+$ males, however, there is an extreme enhancement of the recessive cos phenotype to give a pupal lethal phenotype, with pharate adults expressing the pattern duplications typical of cos^{7} flies. In addition, all *fu* alleles are lethal when hemizygous males carry the cos^{13} allele.

humilis: Mutant alleles of *humilis* are either recessive lethal or recessive female-sterile. Eight alleles of this locus have been studied. Five, hum^{1}, hum^{2}, hum^{3}, hum^{4} and hum^{8}, are completely lethal when heterozygous with *Df(2R)ST1*, but give escapers when heterozygous with each other (Table 6). Escaper flies are tiny with disproportionately short legs and wings. The hum^{1} chromosome is female sterile when homozygous or when heterozygous with deletions that include *hum*. It also carries a maternal effect segmentation mutation that causes embryonic shortening and the loss of thoracic segments in embryos from homozygous hum^{1} females. This defective segmentation phenotype is not shown in embryos from the cross of hum^{1}/ Df(*2R*)*NCX11* females to homozygous hum^{1} males or in crosses with any of the other *hum* alleles and so is unlikely to map to the *hum* locus.

Three viable but female sterile alleles of *humilis*, hum^{fs5}, hum^{fs6} and hum^{fs7} were recovered in a screen for chromosomes that were sterile in females heterozygous for hum^{1}. These sterile alleles are viable, but female sterile, when heterozygous with deletions of the *hum* locus and when heterozygous either with each other or with lethal *hum* alleles; the flies are otherwise wild-type. These chromosomes are all homozygous lethal, presumably due to the presence of additional lethal mutations on the second chromosome.

In a three-factor cross the hum^{1} mutation was mapped by recombination between *l(2)43Bd* and *so* (Table 3). Since *Df(2R)NCX9* and *Df(2R)NCX11* complement lethal *hum* alleles for viability, but are so^{-},

TABLE 6

Viability of *humilis* heterozygotes; numbers of Cy⁺ flies from crosses between alleles balanced over *CyO*

	hum^{1}	hum^{2}	hum^{3}	hum^{4}	hum^{8}	hum^{fs5}
hum^{1}	—	2/566	1/595	1/502	ND	ND
hum^{2}		—	2/650	3/508	0/306	99/218
hum^{3}			—	3/738	ND	ND
hum^{4}				—	ND	ND
hum^{8}					—	132/457

Escapers are very small flies, with short legs and wings. When heterozygous with *Df(2R)ST1* all alleles are lethal ($n > 2,000$ *CyO* progeny).

TABLE 7

The viabilities of *so* alleles *inter se* and with *so*⁻ deletions; numbers of non-Cy progeny from crosses between mutations balanced over *CyO*, except for those involving so^1 and so^2, which were homozygous

	so^1	so^2	so^3	so^4	so^5	so^6
so^-	197/477	133/308	0/1324	0/1139	136/437	0/205
so^1	—	ND	121/239	181/320	128/207	59/184
so^2		—	151/215	136/169	99/198	81/166
so^3			—	0/171	67/209	0/357
so^4				—	72/241	0/286
so^5					—	61/161

TABLE 8

Viabilities of *Drl* with mutations of *so*; number of non-Cy progeny between crosses of *Drl* to *so*/*CyO* or so homozygotes (so^1, so^2)

	Drl
so^1	48/106[a]
so^2	79/123[b]
so^3	0/181
so^4	0/173
so^5	27/356[c]
so^6	0/108

[a] Eyes absent or very small, some eye outgrowths, no ocelli.
[b] Eyes absent or very small, ocelli reduced in size, but present.
[c] Eyes very small, ocelli reduced in size, but present.

their breakpoints lie between *hum* and *so* and the *hum* lethality maps to the left of *so*. These deletions, however, fail to complement the sterility of hum^1 and hum^{fs5} females. Thus, the female sterility maps distal to the lethal function. All *so* alleles are fertile in hum^1/*so* females, so that the distal, female-sterile, function of *hum* is distinct from the *so* locus. However, *Drl* and *Drl*-revertant chromosomes fail to complement the female sterility of the hum^1 and hum^{fs5} chromosomes (see below). The *Drl* chromosome may, therefore, carry a lesion affecting the distal, female-sterile, function of the *hum* locus, in addition to its *so* mutation.

***Droplet* is a gain of function allele of *sine oculis*:** The most obvious phenotypes of flies mutant for *sine oculis* are the loss of their ocelli and partial or complete loss of their eyes. Both viable and recessive lethal alleles of *so* have been recovered in this study (Table 7). Overlapping deletions that include the *so* locus are lethal, *e.g.*, *Df(2R)NCX9* or *Df(2R)NCX11* heterozygous with *Df(2R)sple-D2*, *Df(2R)sple-J1* or *Df(2R)sple-J2* (totals summed over all the *sple* deletions with *Df(2R)NCX9* and *Df(2R)NCX11* respectively being 0/618 and 0/401). All alleles of *so* that are lethal with so^- deletions are also lethal *inter se*.

Three alleles of *so* (so^3, so^4 and so^6) have been recovered in F_2 screens of EMS-treated chromosomes over *Df(2R)ST1* (so^3, so^4) or *Df(2R)pk-78k* (so^6). In addition a weak, viable allele (so^5) was recovered in an F_1 EMS screen against *Df(2R)NCX9*.

so^1 is a spontaneous mutation. About 80% of homozygous so^1 adults completely lack eyes and ocelli. In the remaining flies, one or both eyes are reduced to isolated ommatidia in the posterior region of the normal eye. About 5% of the eyes have outgrowths of ommatidial-like cells, these tend to be at the posterior region of the eye. In place of corneal tissue, a disorganized patch of bristle-bearing cuticle is formed. It seems likely, therefore that the eyeless phenotype may be associated with an alteration in cell fate.

The so^1 mutation is not completely recessive as about 10% of eyes in so^1/flies show ectopic bristles or limited corneal outgrowths. so^1/so^3 or so^1/so^4 flies have eyes of intermediate size, slightly reduced ocelli and 90% of the flies show corneal outgrowths. Similar phenotypes are seen in so^1/*Df(2R)so*⁻ flies but not in +/*Df(2R)so*⁻ flies. Since partial dominance and corneal outgrowths of so^1 are not seen in *Df(2R)so*⁻/+ flies, so^1 is not a simple hypomorphic mutation. Either so^1 has acquired a new function (it is a neomorph) or there is an unlinked dominant enhancer of the mutant phenotype in the so^1 stock. The so^1 chromosome has reduced viability at high temperatures (Ransom 1980), but is not lethal (Lindsley and Zimm 1992).

A hypomorphic allele, so^2, is a spontaneous partial reversion of so^1 (Lindsley and Zimm 1992). Homozygous so^2 flies have normal, or slightly reduced, eyes but no ocelli. *Df(2R)so*−/so^2 flies have small rough eyes and no ocelli. In so^2/so^1 flies the eyes are of intermediate size and the dominant so^1 phenotype of corneal disruption and outgrowths is enhanced, so that 50% of eyes are affected to a variable extent. A third viable allele, so^5, gives small rough eyes and reduced ocelli in *Df(2R)so*⁻/so^5, so^3/so^5 and so^4/so^5 flies. The so^5 mutation, however, complements the so^1 and so^2 mutations completely, *i.e.*, so^1/so^5 and so^2/so^5 flies are indistinguishable from so^1/+ and wild-type flies, respectively.

The dominant EMS-induced *Drl* mutation causes an extreme reduction in the size of the eye when heterozygous, similar to that of homozygous so^1 flies. However, the ocelli are not severely reduced in *Drl*/+ flies. *Drl* is lethal when homozygous, when heterozygous with so^- deletions or with lethal alleles of *so* (Table 8). In *Drl*/so^1 flies both the eyes and ocelli are very reduced. *Drl* flies with three copies of so^+ (*e.g.*, *Drl/Dp(2;2)Sco*^{rvIL}*TE35A-14*^{R}; *Dp(2;3)P32*/+) have a weak eye phenotype that is approaching wild-type. *Drl* flies with two copies of so^+ (*e.g.*, *Drl/Dp(2;2)Sco*^{rvIL}*TE35A-4*^{R} or *Drl/Dp(2;2)Sco*^{rvIL}*TE35A-14*^{R}) have a moderate eye phenotype, less severe than that of *Drl*/+. The suppression of the phenotype of

Drl by extra copies of so^+ suggests that *Drl* may be an antimorphic allele of *so*.

A number of phenotypic revertants of *Drl* were recovered in X-ray screens (see above). Twenty-six of these are phenotypically wild-type in $Drl^{rv}/+$ flies. Three of the revertant chromosomes, however, are only partial and retain mutations giving a reduction in eye size ($T(2;3)Drl^{rv1}$, Drl^{rv8} and Drl^{rv27}, Table 1). All except four of the revertants that are associated with chromosome aberrations have a breakpoint close to, or delete, 43B1-3.

The majority of revertants, including all those that carry cytologically visible deletions in 43B-C (Table 1), give a typical so^1 phenotype, with neither eyes nor ocelli, when heterozygous with the so^1 chromosome. That is to say, these revertants behave as so^- chromosomes. Nine revertants, however, give either normal eyes and reduced ocelli (Drl^{rv4}, Drl^{rv10}, Drl^{rv11} and Drl^{rv14}) or reduced eyes and reduced (Drl^{rv6}, Drl^{rv8} and Drl^{rv31}) or absent ($T(2;3)Drl^{rv1}$, $Tp(2;2)Drl^{rv23}$) ocelli in Drl^{rv}/so^1 flies. Thus, the eye and ocellar phenotypes of *so* are genetically separable.

All the revertant chromosomes, including the four (*rv4*, *rv10*, *rv11* and *rv14*) on cytologically normal chromosomes that give normal eyes when heterozygous with so^1, remain lethal when heterozygous with an so^- deletion. Thus, although some of the revertants almost completely complement the visible phenotype of so^1, the recessive lethality of the *Drl* mutation is retained.

fs(2)43Ei: A single EMS-induced allele of the novel female sterile, *fs(2)43Ei*, was recovered. In addition to the female sterility, homozygous *fs(2)43Ei* flies have a disarranged pattern of tergite bristles. The bristle phenotype and female sterility are also shown by *Df(2R)ST1/Df(2R)NCX10* flies, which are overlapping deletions that remove the *fs(2)43Ei* and *cn* loci.

DISCUSSION

A set of 175 mutations, including 46 chromosomal deletions define 27 complementation groups within the 43A-E interval. In the proximal part of this region, it is likely that most of the complementation groups have been identified, as multiple alleles of most have been recovered (Table 2). In the distal region, however, several loci are defined by only single alleles and additional complementation groups may remain to be discovered.

Mutant alleles of *necrotic* result in an early adult death, within 24 hr of eclosion the flies develop melanotic spots. The complementation seen between weak and strong *nec* alleles is peculiar, since it indicates that the strong alleles retain some *nec* function. Nevertheless, the strong alleles of *nec* have, when heterozygous with nec^- a similar phenotype to that seen in nec^- homozygotes. A pattern of complementation between amorphic and hypomorphic alleles similar to that seen at *nec* has been described at the *engrailed* (*en*) locus (GUBB 1985). The en^1 mutation gives a strong phenotype when heterozygous with a deletion, but when heterozygous with amorphic, lethal alleles the $en^1/l(2)en$ flies are almost wild-type. This pattern of complementation does not reflect residual en^+ activity in the lethal alleles (CONDIE and BROWER 1989).

If some of the amorphic *nec* alleles do correspond to complete lack of nec^+ activity, then the partial complementation between amorphic and hypomorphic alleles requires an alternative explanation. The formal possibilities are either that the deletion chromosomes remove a related gene that acts as a suppressor of *nec*, or that the level of nec^+ activity is modulated by feedback repression that is sensitive to the number of copies of the locus.

Given that the *nec* and *pk* alleles on the $In(2R)nec^3pk^3$ and $In(2R)nec^5pk^5$ chromosomes are hypomorphic, the double mutations cannot result from small deletions that include both loci being associated with the inversion breakpoints. A likely explanation is that the double mutant phenotypes result from position effect variegation, which can spread linearly along a chromosome from a heterochromatic breakpoint (see HENIKOFF 1990 for a recent review); both the $In(2R)nec^3pk^3$ and $In(2R)nec^5pk^5$ have breakpoints that juxtapose the 43A region to the centric *2R* heterochromatin (Table 1).

There is a complex pattern of complementation between *pk* and *sple* alleles. In classical genetic terms these correspond to two adjacent genes that can be separated either by recombination (GUBB and GARCÍA-BELLIDO 1982) or by aberration breakpoints. Moreover, there is complete complementation between single alleles at these two loci. The two mutant phenotypes are similar, however, although they affect reciprocal regions of the body. The striking observation is that double-mutant alleles, which fail to complement both single *pk* and single *sple* alleles, can be recovered at a high frequency.

These three classes of mutation, two single and one double, might be affecting a single, complex, transcription unit. An alternative possibility is that a cluster of transcription units controlling related genetic functions is involved. Distinguishing these possibilities must await the molecular analysis of these genes.

The *sine oculis* gene, hitherto known for its visible phenotypes (the loss of the eyes and ocelli) is shown to be a vital locus. Not only are deletions that only, as far as is known, overlap the *so* locus lethal, but also lethal EMS-induced *so* alleles are not uncommon.

During pupal development, the so^1 phenotype is associated with extensive cell death in the optic lobes (FISCHBACH and TECHNAU 1984). This cellular degen-

eration results from an increase in death of the precursor neurons compared with normal development (FISCHBACH 1983) and leads to a reduced number of axons in the anterior optic tract (FISCHBACH and LYLY-HÜNERBERG 1983). A discrete group of lamina precursor cells fail to enter their final S phase in the brains of *so*[1] larvae (SELLECK *et al.* 1992). A similar failure in the final divisions posterior and anterior to the morphogenetic furrow during imaginal disc development might result in incorrect specification of the presumptive corneal cells.

The *Drl* mutation is a dominant antimorphic allele of *so*. This is shown by the interactions between *Drl* and *so* mutations and by the properties of *Drl* revertants. *Drl* fails to complement the sterility of female sterile mutations of the adjacent *hum* locus and may be associated with a small deletion or other chromosome aberration or be genetically complex. The complex pattern of complementation between the *Drl*, *Df(2R)NCX9* and *Df(2R)NCX11* chromosomes and the different classes of *hum* and *so* alleles suggests that there may be several functionally related transcripts in the *hum-so* interval. In particular, there appear to be lethal and viable morphogenetic functions at the *so* locus that are partially complementary.

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