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Enhancement of Overgrowth by Gene Interactions in *lethal(2)giant discs* Imaginal Discs From *Drosophila melanogaster*

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ABSTRACT

Recessive lethal mutations of the *lethal(2)giant discs* (*l(2)gd*) and *lethal(2)fat* (*l(2)ft*) loci of *Drosophila melanogaster* cause imaginal disc hyperplasia during a prolonged larval stage. Imaginal discs from *l(2)ft l(2)gd* or *G l(2)gd* double homozygotes show more extensive overgrowth than in either single homozygote, and double homozygous *l(2)ft l(2)gd* mitotic clones in adult flies show much more overgrowth than is seen in clones homozygous for either *l(2)gd* or *l(2)ft* alone. *dachsous* (*ds*) also acts as an enhancer of *l(2)gd*, producing dramatically overgrown discs and causing failure to pupariate in double homozygotes. The *comb gap* (*cg*) mutation, which also interacts with *ds*, greatly enhances the tendency of imaginal discs from *l(2)gd* larvae to duplicate as they overgrow. If *l(2)gd* homozygotes are made heterozygous for *l(2)ft*, then several discs duplicate, indicating that *l(2)ft* acts as a dominant enhancer of *l(2)gd*. *l(2)ft* also acts as a dominant enhancer of *l(2)gd*, and conversely *l(2)gd* acts as a dominant modifier of *l(2)ft*. The enhancement of overgrowth caused by various mutant combinations is accompanied by changes in expression of Decapentaplegic and Wingless. These results show that tumor suppressor genes act in combination to control cell proliferation, and that tissue hyperplasia can be associated with ectopic expression of genes involved in pattern formation.

LOSS of cell proliferation control is a fundamentally important aspect of tumorigenesis and often occurs independently of other defects such as loss of cell contact and differentiation. In fact, the formation of tumors is often preceded by simple overproliferation or hyperplasia (*e.g.*, MIRSALIS and STEINMETZ 1990; KLEIN-SZANTO 1991; MCDONNELL and KORSMEYER 1991; GAZDAR 1994; MECKE *et al.* 1994; LEVINE 1995; TAN *et al.* 1995). Hyperplastic cell populations then accumulate other mutations and perturbations, leading to the more malignant phase of tumorigenesis. Full tumorigenesis is achieved when cells are able to leave the primary tumor and metastasize to other tissues, where they can establish secondary tumors (MORGAN-PARKES 1995).

Drosophila provides a convenient model system for cancer studies. Over 60 genes have been identified in this organism where loss-of-function mutations lead to excessive cell proliferation in the imaginal discs, brain, embryo, gonads, or hematopoietic system (GATEFF 1994; WATSON *et al.* 1994). We refer to these genes as tumor suppressor genes. Mutations in some of these genes give rise to hyperplasia, whereas mutations in others can give rise directly to malignancy (GATEFF 1978) and/or invasiveness (TIMMONS *et al.* 1993). These mutations provide an excellent opportunity to study tumorigenesis at the molecular, genetic, cell biological and developmental levels.

In this article we have investigated interactions between two genes that are required for cell proliferation control in the imaginal discs of the larva. Recessive lethal mutations in these two genes, called *l(2)gd* and *l(2)ft*, cause hyperplastic imaginal disc overgrowth; the discs maintain their epithelial structure as they overgrow, and they retain the ability to differentiate at least some cuticular structures (BRYANT and SCHUBIGER 1971; BRYANT and LEVINSON 1985; BRYANT *et al.* 1988; MAHONEY *et al.* 1991). We have also investigated interactions between *l(2)gd* and two additional genes that are required for normal pattern formation but are not involved in general cell proliferation control, namely *dachsous* (*ds*) and *comb gap (cg)*. The *l(2)ft* and *ds* genes encode large, transmembrane cadherin-like molecules (MAHONEY *et al.* 1991; CLARK *et al.* 1995), but the products encoded by *l(2)gd* and *cg* are not known. The results reveal several examples of dramatic phenotypic interactions between these genes, supporting the possibility that multiple genetic mutations can contribute synergistically to tumorigenesis (VOGELSTEIN and KINZLER 1993; BERNS *et al.* 1994). Enhanced overgrowth is correlated with increased patterning defects, and increased ectopic expression of certain genes is important for patterning. Such a connection between overgrowth and patterning defects suggests that mutations in some tumor suppressor genes might also affect the spatial ex-

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pression of genes important in patterning (AGRAWAL *et al.* 1995; BURATOVICH and BRYANT 1995; MUKHERJEE *et al.* 1995).

MATERIALS AND METHODS

Stocks: *ds^{38k}/dp Cy In(2LR) b pr* came from the Indiana University stock center. *y w; Dp(2;1)sc^{19}, sc^{19} M(2)z/SM5* (*SM5* is an abbreviation for *In(2LR)Sm5, al^2 Cy lt^v cn^2 sp^2*) was provided by A. GARCIA-BELLIDO (Universidad de Madríd). *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2L)Cy, S^2 Cy,* and *cg c/In(2LR)U* were obtained from the Bowling Green Stock Center (North Carolina). *wg^{NZ}/CyO* (*CyO* is an abbreviation for *In(2LR)O Cy dp^{lvl} pr cn^2*) came from G. STRUHL (Columbia University). *dpp^{d5}/CyO* came from W. GELBART (Harvard University). The stock containing the *dpp-lacZ* transgene *cn BS3.0* was obtained from R. BLACKMAN (Indiana University). The stocks that were maintained in the laboratory and used in this study are as follows: *y; l(2)gd^1 a px or/BcGla* (*BcGla* is an abbreviation for *In (2LR)Gla, GlaBc*), *l(2)gd^1 a px or/BcGla, l(2)ft^{fd} dp^{ovn} or/CyO, l(2)ft^{a13} or/CyO, y; l(2)ft^{fd} dp^{ovn} or/CyO, y; l(2)ft^{a13} or/CyO, y; l(2)ft^{fd} dp^{ovn} l(2)gd^1 a px or/CyO, al G/BcGla, al G l(2)gd^1 a px or/BcGla, l(2)gd^{d7}/BcGla.* All other stocks were made by recombination.

Larvae from each cross were obtained from 4–6-hr egg collections at 25°, from 25 males and 25 females in a standard vial, that gave a larval density of about 60 larvae/cm^2 of food surface. Larval ages are given in days after egg laying. Unfixed, unstained imaginal discs were dissected and photographed in Chan and Gehring's buffer (CHAN and GEHRING 1971), using brightfield microscopy. *l(2)ft^{fd} l(2)gd^1/l(2)gd^1* larvae were obtained by crossing *l(2)gd^1 a px or/BcGla* to *y; l(2)ft^{fd} dp^{ovn} l(2)gd^1 a px or/CyO,* and *l(2)ft^{fd} l(2)gd^1/l(2)ft^{fd}* larvae were obtained by crossing *y; l(2)ft^{fd} l(2)gd^1 a px or/CyO* to *l(2)ft^{fd} or/CyO.* These and *y; l(2)ft^{fd} dp^{ovn} l(2)gd^1 a px or* larvae were identified by their colorless Malpighian tubules due to the *or* marker. In all other crosses the mutant larvae were identified by the absence of the *black cells* phenotype caused by *Bc* carried by the *BcGla* balancer chromosome.

Mitotic clones: By utilizing the *sc^{19}* translocation, in which the *$yellow^+$* (*y^+*) gene from the *X* chromosome has been relocated to the second chromosome, *y* mitotic clones in a *y^+* background were made by irradiating the progeny of the following crosses: *y; l(2)gd^1 a px or/BcGla* females were crossed to *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2L)Cy, S^2 Cy* males to yield *y; l(2)gd^1* clones; *y; l(2)gd^1 a px or/BcGla* females were crossed to *y w; Dp(2;1)sc^{19}, sc^{19} M(2)z/SM5* males to produce *y; l(2)gd^1 M^+* clones in a *Minute* background; *y; l(2)ft^{fd} dp^{ovn} or/CyO* females were crossed to *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2L)Cy, S^2 Cy* males to make *y; l(2)ft^{fd}* clones; *y: l(2)ft^{fd} dp^{ovn} or/CyO* females were mated to *y w; Dp(2;1)sc^{19}, sc^{19} M(2)z/SM5* males to give *y; l(2)ft^{fd} M^+* clones in a *Minute* background; *y; l(2)ft^{fd} dp^{ovn} l(2)gd^1 a px or/CyO* females were mated to *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2L)Cy, S^2 Cy* males to produce *y; l(2)ft^{fd} l(2)gd^1* clones; *y; l(2)ft^{fd} dp^{ovn} l(2)gd^1 a px or/CyO* females were mated to *y w; Dp(2;1)sc^{19}, sc^{19} M(2)z/SM5* males to give *y; l(2)ft^{fd} l(2)gd^1 M^+* clones in a *Minute* background; *y; ds^{38k} l(2)gd^1/CyO* females were mated to *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2L)Cy, S^2 Cy* males to produce *y; ds^{38k} l(2)gd^1* clones; *y w* females were mated to *y ac; Dp(2;1)sc^{19}, sc^{19}/In(2-L)Cy, S^2 Cy* and *y w; Dp(2;1)sc^{19}, sc^{19} M(2)z/SM5* males to make *y w* and *y w; M^+* control clones, respectively. The parents were allowed to lay eggs for 4–8 hr. The timed progeny were irradiated at various times after egg laying, with 1000 rads of gamma radiation from a ^{137}Cs source. Adult flies from these crosses were then fixed in 70% ethanol. Clones on the legs, head or body were photographed wet, or the appendage was dissected from the fly, placed in 100% ethanol for 5 min, mounted on a slide in Euparol, and photographed. The area of each mitotic clone was determined by first tracing the outline of the clone, and then measuring the area inside the outline with a compensating polar planimeter.

Disc staining: The protocols of PHILLIPS *et al.* (1990) were used for X-Gal staining and *in situ* hybridization. To stain discs with antibodies to bacterial β-galactosidase, we used a mouse monoclonal anti-β-galactosidase antibody (Promega) and detected this with a goat anti-mouse secondary antibody conjugated to horseradish peroxidase (Zymed). The protocol of CAMPBELL *et al.* (1993) was used to visualize horseradish peroxidase-conjugated antibodies.

RESULTS

Phenotypes of *l(2)gd* and *l(2)ft*

***l(2)gd*:** Alleles of *l(2)gd* form a series of decreasing severity in the homozygote, based on the frequency with which the overgrowing imaginal discs show pattern duplications, with *l(2)gd^{d7}* being the most and *l(2)gd^1* the least severe (Table 1). In *l(2)gd^1* the wing pouch of the wing disc extends distally and overgrows as a flat sheet (Figure 1, c–f; compare with Figure 1, a and b). The third leg disc duplicates the end knob as it overgrows (BRYANT and SCHUBIGER 1971). The imaginal rings in the gut and salivary glands also overgrow in this mutant (BRYANT and LEVINSON 1985).

When no mitotic clones of *l(2)gd^1* (Table 2) were observed among 600 legs, it seemed likely that the mutant clones were simply not growing as fast as the *l(2)gd^+* cells, and, therefore, were lost by cell competition (SIMPSON and MORATA 1981). To test this possibility, we induced *l(2)gd^1* mitotic clones in a *Minute* background, which gives the *l(2)gd^1* cells a growth advantage since they are *$Minute^+$* (MORATA and RIPOLL 1975). *l(2)gd^1 M^+* clones do not form outgrowths but are recognizable by *y* and/or missing bristles, and occasionally, empty or duplicated sockets (Figure 2A). A markedly reduced bristle number is also seen in *l(2)gd^1* pharate adults and in the derivatives of *l(2)gd^1* imaginal discs that have been transplanted into wild-type larvae for metamorphosis (BRYANT and SCHUBIGER 1971). Imaginal discs from *l(2)gd^1* larvae also show no expression of the bristle patterning genes *achaete* and *scute* (M. A. BURATOVICH, unpublished results). As bristle precursor cells formed late in imaginal-disc development (HARTENSTEIN and POSAKONY 1989; HUANG *et al.* 1991; USUI and KIMURA 1993), the reduction or absence of bristles in *l(2)gd* might indicate an inability to proceed with the later stages of disc development. This idea is supported by the observation that *l(2)gd* discs fail to express several genes that are normally expressed later in development (*e.g., bric-a-brac;* M. BURATOVICH and P. BRYANT, unpublished results).

***l(2)ft*:** In *l(2)ft^{fd}*, the imaginal discs do not show any obvious duplication of structures (Figure 1, g–j), and they overgrow in multiple directions (BRYANT *et al.* 1988). Heterozygotes for the dominant allele *G* (MOHR 1929) have outstretched wings, and *G* homozygotes, like

TABLE 1

Frequency of imaginal disc duplication in combinations of overgrowth mutants

Genotype	Eye-antenna	First leg	Second leg	Third leg	Wing	Haltere	Male genital	Female genital
$l(2)gd^{d7}$	43	0	63	98	48	77	7	12
$l(2)gd^{1}$	0	0	0	88	0	0	0	0
$l(2)ft^{fd}$ $l(2)gd^{1}/l(2)gd^{1}$	83	5	85	100	91 (10)	88	2	8
$l(2)ft^{fd}$	0	0	1	0	0	0	0	0
$l(2)ft^{fd}$ $l(2)gd^{1}/l(2)ft^{fd}$	45	0	3	0	38	0	0	0
$l(2)ft^{G}$ $l(2)gd^{1}/l(2)gd^{1}$	15	0	0	0	83	100	92	88
$l(2)ft^{G}$ $l(2)gd^{1}$	80	0	0	0	92	89	100	100
$l(2)ft^{fd}$ $l(2)gd^{1}$	67	32	0	0	0	66	8	16
ds^{38k} $l(2)gd^{1}$	82	0	0	19	0	100	22	40
ds^{38k} $l(2)gd^{1}/l(2)gd^{1}$	72	6	82	100	85	82	0	0
$l(2)gd^{1}cg$	88	0	84	100	75	78	0 (12)	58 (12)

[a] In all cases $N = 100$. Unless otherwise indicated by number of days in parentheses, duplication frequencies were determined at 9 days.

$l(2)ft^{fd}$ homozygotes, show hyperplastic overgrowth of the imaginal discs (data not shown).

Mitotic clones of $l(2)ft^{fd}$ or $l(2)ft^{a13}$ make outgrowths that are, unlike $l(2)gd^{1}$ clones, covered with bristles (Figure 2, B and C), and some form vesicles with internally facing bristles (Figure 2B). Internal vesicles are also found in $l(2)ft^{fd}$ pharate adults (Bryant *et al.* 1988), consistent with a defect in cell adhesion. *l(2)ft* clones show an apparent increase in the number of bristles, and $l(2)ft^{a13}$ imaginal discs show increased expression of the bristle formation gene *achaete* (M. A. Buratovich, unpublished results).

Synergistic effects on growth

Double homozygotes of *l(2)ft l(2)gd* or *G l(2)gd* show novel disc phenotypes: Imaginal discs from $l(2)ft^{fd}$ $l(2)gd^{1}$ double homozygotes also show extensive overgrowth (Figure 1, k–n). Wing discs of this genotype bend at the hinge region and show many extra folds that probably result from excess proliferation. Discs from these larvae at 4–5 days are smaller than discs from wild-type, $l(2)ft^{fd}$, or $l(2)gd^{1}$ homozygotes at 4–5 days, but they continue growing for longer and eventually outgrow discs of these other genotypes. The leg discs are greatly overgrown, sometimes approximating the size of a wild-type wing disc, and, with the exception of the first leg disc, show no apparent signs of duplication (Table 1). The haltere disc is also quite overgrown and shows occasional duplication of the capitellum pouch. The eye-antenna disc is massively overgrown and becomes the largest disc in $l(2)ft^{fd}$ $l(2)gd^{1}$ larvae. It shows a variety of duplications and triplications of both the antenna knob and the retinal field. Both male and female genital discs duplicate and show many extra folds. $l(2)ft^{fd}$ $l(2)gd^{1}$ and G $l(2)gd^{1}$ double homozygous larvae rarely live longer than 9d, and they never make pharate adults. Imaginal discs from double homozygous G $l(2)gd^{1}$ larvae (Figure 1, o–r) resemble those of $l(2)ft^{fd}$ $l(2)gd^{1}$.

$l(2)ft^{fd}$ $l(2)gd^{1}$ mitotic clones produce very few bristles (Figure 2D). Sometimes the clone contains some empty bristle sockets, but more often bristles and sockets are completely missing. The cuticle of mitotic clones induced on the head or notum is usually twisted and rough (Figure 2D). Clones induced on the wings interfere with vein formation. Internal vesicles are seen, but unlike those observed in $l(2)ft^{fd}$, internal vesicles formed from $l(2)ft^{fd}$ $l(2)gd^{1}$ clones do not have have bristles (data not shown). $l(2)ft^{fd}$ $l(2)gd^{1}$ clones are larger than either $l(2)ft^{fd}$ or $l(2)gd^{1}$ clones in both a *Minute* (Table 2) and a *Minute*$^{+}$ (Table 3) background (compare Figure 2D with A–C). Thus, even though *l(2)gd* causes cells to grow so slowly that they are lost by cell competition, and

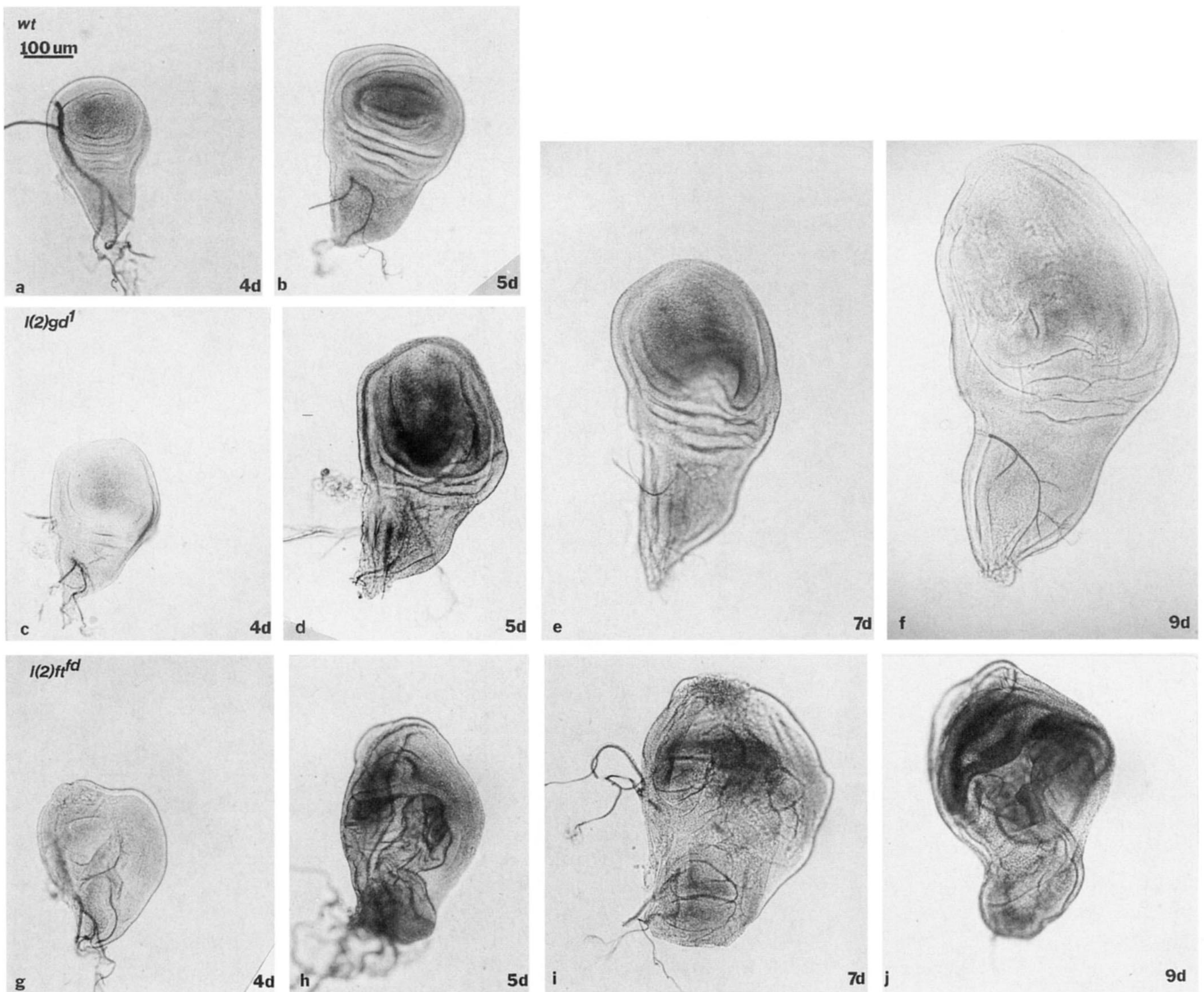

FIGURE 1.—Wing imaginal discs from (a and b) wild-type larvae, (c–f) $l(2)gd^1$ *a px or* larvae, (g–j) $l(2)ft^{fd}$ dp^{ovn} *or* larvae, (k–n) $l(2)ft^{fd}$ dp^{ovn} $l(2)gd^1$ *a px or* larvae, (o–r) *al G* $l(2)gd^1$ *a px or* larvae. Discs shown as follows: (a) 4 days, (b) 5 days, (c) 4 days, (d) 5 days, (e) 7 days, (f) 9 days, (g) 4 days, (h) 5 days, (i) 7 days, (j) 9 days, (k) 4 days, (l) 5 days, (m) 7 days, (n) 9 days, (o) 4 days, (p) 5 days, (q) 7 days, (r) 9 days.

subsequently not recovered in a wild-type background, doubly homozygous *l(2)ft l(2)gd* cells grow faster than both wild-type and *l(2)ft* cells.

ds^{38k} is an enhancer of *l(2)gd*: Like *l(2)ft, dachsous* encodes a giant member of the cadherin superfamily. *dachsous* mutations, however, cause defects during morphogenesis and do not appear to affect imaginal disc morphology or growth (CLARK *et al.* 1995). Wing discs from ds^{38k} $l(2)gd^1$ homozygous larvae (Figure 3, a–d) are large and quite variable in their morphology. A series of frill-like folds on the edges of the discs is characteristic of this genotype. The leg discs from ds^{38k} $l(2)gd^1$ larvae are also much larger and thicker than those from $l(2)gd^1$ larvae of the same age. The haltere disc duplicates, triplicates, or quadruplicates. The eye-antenna discs show a pair of large knob-like structures at the anterior end of the antenna field. These knobs can first be seen at ~7 days, and they appear next to the antenna knob. At 8 days, the knob has separated from the original antenna knob and has become displaced anteriorly, and by 9 days, it has split into a pair of knobs. These knobs are probably presumptive antennae (Table 1). The genital discs show many extra lobes (data not shown). These animals usually die in the larval or early pupal stage, and never reach the pharate adult stage.

ds^{38k} $l(2)gd^1$ clones form protrusions, due to abnormal morphogenesis, that might result from an excess of cells (data not shown). Since $l(2)gd^1$ does not form protruding mitotic clones, we conclude that ds^{38k} enhances the overgrowth phenotype of $l(2)gd^1$.

Effect of *cg* on the disc phenotype of *l(2)gd*: Wing discs from $l(2)gd^1$ cg^1 larvae show a variety of extra folds and extensive overgrowth (Figure 3, e–h). The second

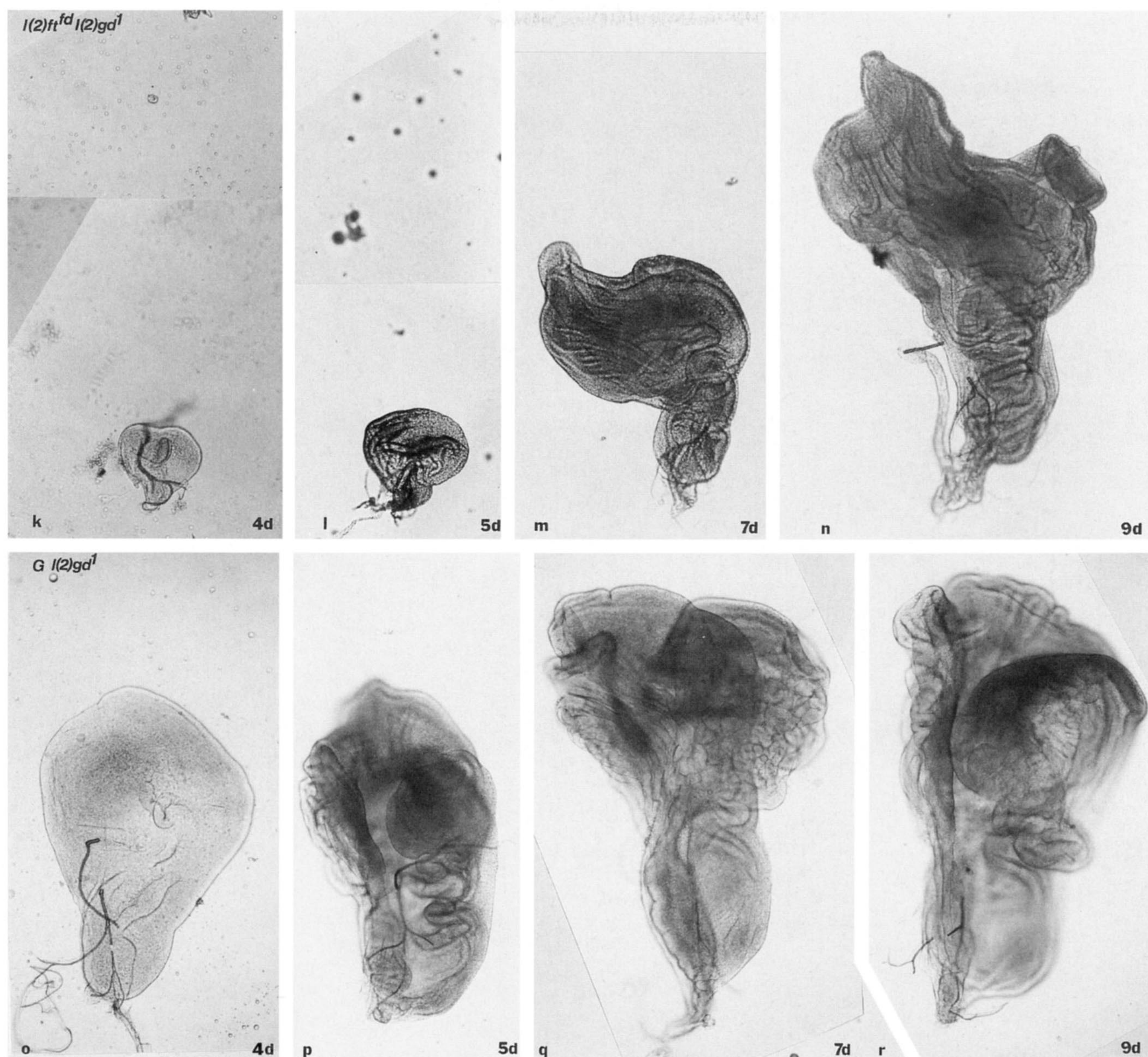

FIGURE 1.—*Continued*

and third leg, haltere, female genital, and eye-antenna discs all show duplication of various structures (Table 1). These animals fail to form pharate adults and die as either larvae or pupae. cg^{1} mutations normally do not affect the imaginal discs.

TABLE 2

Mitotic clone size in a *Minute*$^{+}$ background

Genotype of clone	Area of head clones induced at 50 hr (10^{-2} mm^{2})	*N*
y; l(2)gd^{1}	ND	
y; l(2)ft^{fd}	8.4 ± 3.2	15
y; l(2)ft^{fd} *l(2)gd*^{1}	14.7 ± 3.6	13
y	7.80 ± 2.9	12

Dominant enhancement of growth

***l(2)ft* is a dominant enhancer of *l(2)gd*:** Imaginal discs from $l(2)ft^{fd}$ $l(2)gd^{1}/l(2)gd^{1}$ larvae show consistent duplications of the second and third leg, haltere, eye-antenna and wing discs at 9 days (Figure 4, a–d; Table 1). In contrast, discs from $l(2)gd^{1}/l(2)gd^{1}$ animals show duplication only of the third leg disc, even in larvae as old as 10 days (BRYANT and SCHUBIGER 1971). $l(2)ft^{fd}$ $l(2)gd^{1}/l(2)gd^{1}$ animals never make pharate adults, even though $l(2)gd^{1}/l(2)gd^{1}$ animals can pupariate and form pharate adults (BRYANT and SCHUBIGER 1971). Therefore, *l(2)ft* is a dominant enhancer of *l(2)gd*.

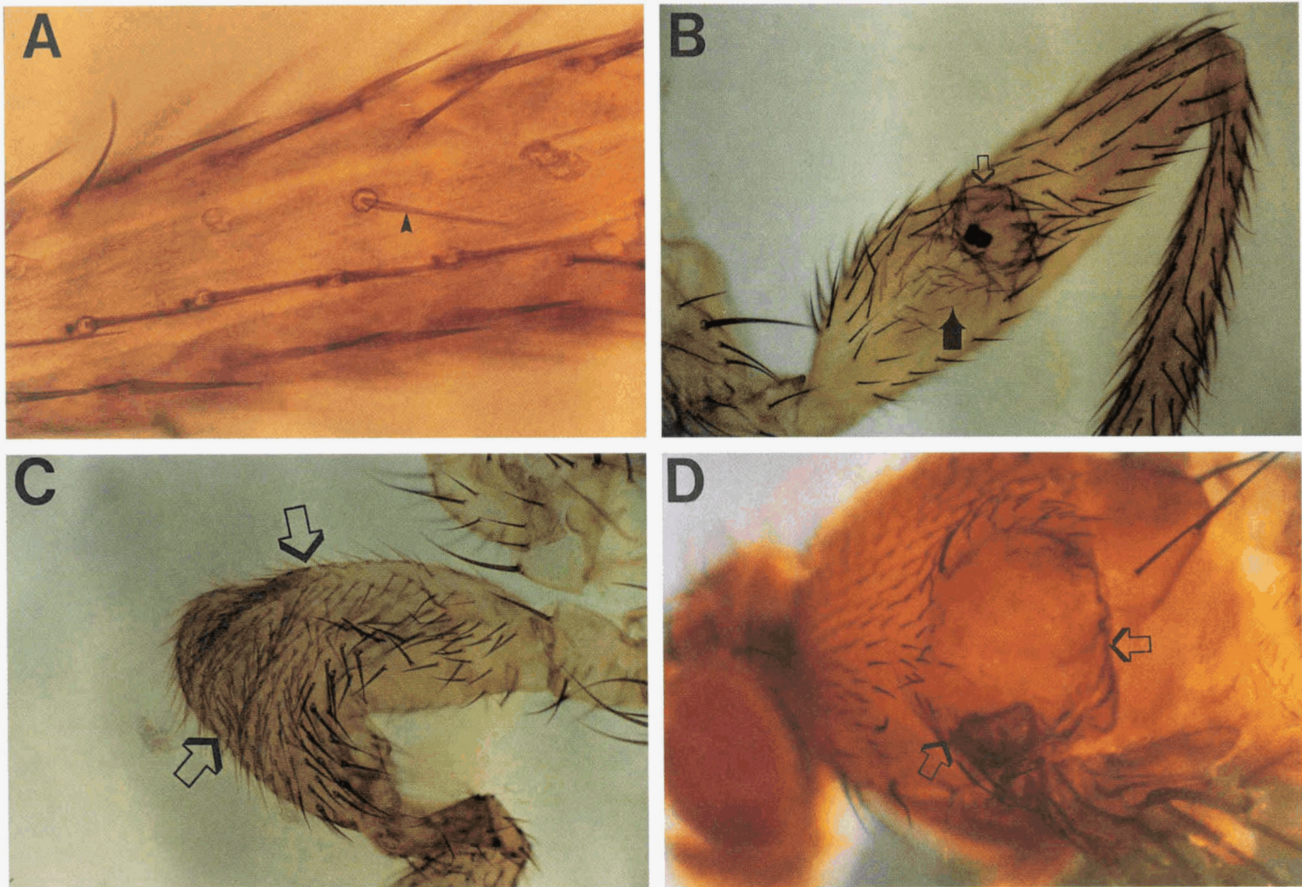

FIGURE 2. Mitotic clones. (A) Leg clone induced in a *y w; Dp(2;1)sc*^{19}*, sc*^{19} *M(2)z/l(2)gd*^{1} *a px or* larva at 50 hr. The arrow marks the yellow bristle. Note the empty bristle sockets. (B) Leg clone induced in a *y ac; Dp(2;1)sc*^{19}*, sc*^{19}*/l(2)ft*^{fd} *or* larva at 45 hr. The closed arrow indicates a yellow bristle, and the open arrow indicates the internal vesicle, which is also marked with yellow bristles. (C) Leg clone induced in a *y ac; Dp(2;1)sc*^{19}*, sc*^{19}*/l(2)ft*^{a13} *or* larva at 49 hr. The overgrowth in the clone has caused the entire leg to bend. The two open arrows indicate the array of yellow bristles that mark the clone. (D) Notal clone induced in a *y ac; Dp(2;1)sc*^{19}*, sc*^{19}*/dp*^{ovn} *l(2)ft*^{fd} *l(2)gd*^{1} *a px or* larva at 49 hr. The two open arrows indicate the area of the clone. Since *l(2)gd* prevents bristle formation, the overgrown area correlates with the area that has lost bristles.

***G* is a dominant enhancer of *l(2)gd*:** Wing discs from *G l(2)gd*^{1}*/l(2)gd*^{1} larvae (Figure 4, e–h) are much larger than *l(2)gd*^{1}*/l(2)gd*^{1} wing discs of the same age (Figure 1f) and show a different morphology. Instead of an overgrown wing disc that grows as a flat sheet of cells, the entire disc is elongated and thickened with several extra folds, much like discs from *l(2)ft* larvae. Thus the introduction of one copy of *G* can change the overgrowth pattern of *l(2)gd* discs such that they resemble *l(2)ft* more than *l(2)gd*. The leg discs are also different in their pattern of overgrowth, compared with *l(2)gd*^{1}*/l(2)gd*^{1}. At 9 days, neither the third leg nor any of the other leg discs show duplication of the end knob (Figure 6, g–j) but the haltere shows duplication and sometimes triplication (Table 1). The genital discs also show several additional lobes, in contrast to *l(2)gd*^{1} genital discs, which simply grow larger than normal (BRYANT and SCHUBIGER 1971). This shows that *G* is also a dominant enhancer of *l(2)gd*.

TABLE 3

Mitotic clone size in a *Minute* background

Genotype of clone	Area of leg clones induced at 90 hr (10^{-2} mm^{2})	*N*
y; l(2)gd^{1} *M*$^{+}$	5.4 ± 2.6	13
y; l(2)ft^{fd} *M*$^{+}$	7.3 ± 2.4	12
y; l(2)ft^{fd} *l(2)gd*^{1} *M*$^{+}$	14.0 ± 3.6	14
y; M^{+}	6.2 ± 2.1	12

ND, not determined.

***l(2)gd* is a dominant modifier of *l(2)ft*:** Wing discs from *l(2)ft*^{fd} *l(2)gd*^{1}*/l(2)ft*^{fd} larvae (Figure 4, i–l) show a distally elongated wing pouch characteristic of *l(2)gd*^{1} rather than *l(2)ft*^{fd} (compare Figure 1, c–f *vs.* 1, g–j). Furthermore, 9-day wing discs show duplication of the wing pouch (Figure 4l) and the eye disc (data not shown), even though neither of these tissues show pattern duplication in *l(2)ft*^{fd} larvae. These changes show that *l(2)gd* is a dominant modifier of *l(2)ft*.

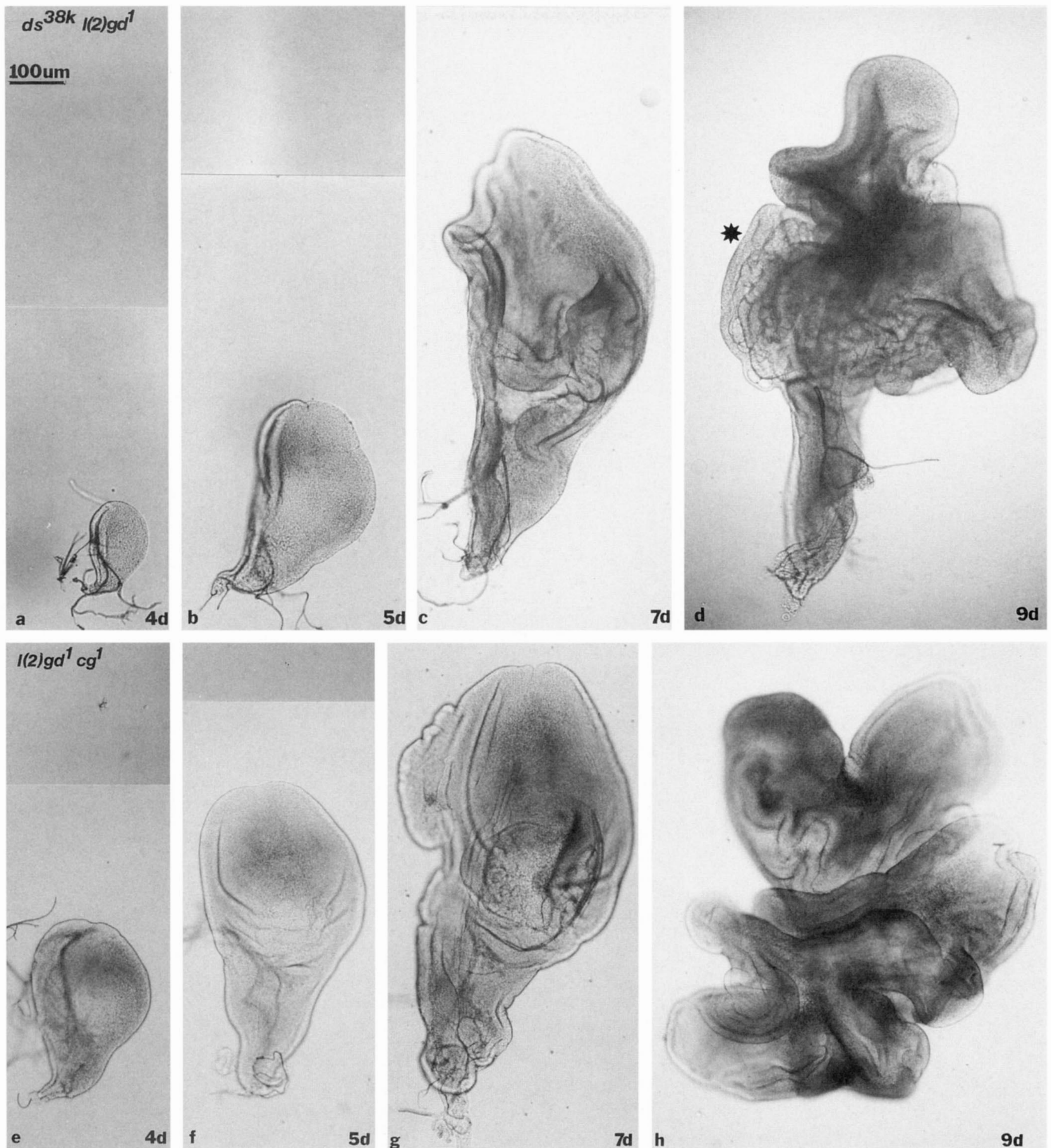

FIGURE 3.—Wing imaginal discs from (a–d) ds^{38k} $l(2)gd^1$ and (e–h) $l(2)gd^1$ *cg* larvae. Discs are shown as follows: (a) 4 days, (b) 5 days, (c) 7 days, (d) 9 days (* marks the frill-like folds) (e) 4 days, (f) 5 days, (g) 7 days, (h) 9 days.

Effects of overgrowth mutations on gene expression

One copy of either $l(2)ft^{fd}$ or ds^{38k} does not change Wg expression in $l(2)gd^1$ discs: Homozygous $l(2)gd^1$ causes dorsal extension of Wg expression in leg discs and distal extension of Wg expression in wing discs (BURATOVICH and BRYANT 1995). Wg expression in imaginal discs from discs from $l(2)ft^{fd}$ $l(2)gd^1/l(2)gd^1$ and ds^{38k} $l(2)gd^1/l(2)gd^1$ larvae is very similar to that of $l(2)gd^1$ larvae (data not shown). Therefore, the enhancement of *l(2)gd* by ds^{38k} and $l(2)ft^{fd}$ is not accompanied by an alteration in Wg expression.

Double homozygotes of *l(2)gd* and *l(2)ft* do not show spreading of Wg expression in leg discs: *In situ* hybridization with a Wg probe to imaginal discs from $l(2)ft^{fd}$ $l(2)gd^1$ larvae show that Wg expression in $l(2)ft^{fd}$ $l(2)gd^1$ leg discs is similar to that of wild-type (Figure 5, a–d;

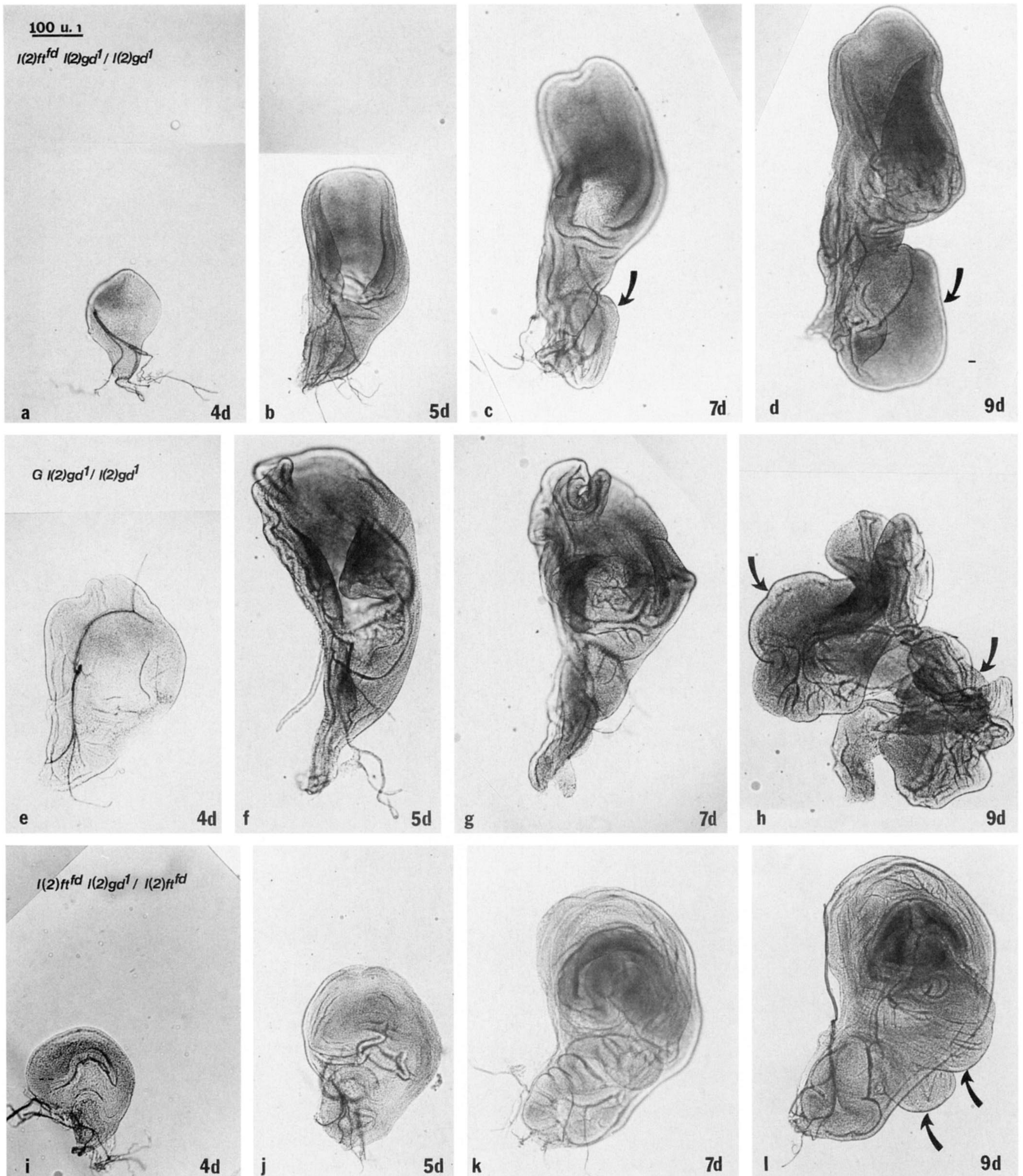

FIGURE 4.—Wing imaginal discs from $l(2)ft^{fd}$ dp^{ovn} $l(2)gd^{1}$ *a px or*/$l(2)gd^{1}$ *a px or* larvae (a–d), *al G* $l(2)gd^{1}$ *a px or*/$l(2)gd^{1}$ *a px or* larvae (e–h), and $l(2)ft^{fd}$ dp^{ovn} $l(2)gd^{1}$ *a px or*/$l(2)ft^{fd}$ dp^{ovn} *or* larvae (i–l). Discs are shown as follows: (a) 4 days, (b) 5 days, (c) 7 days, (d) 9 days, (e) 4 days, (f) 5 days, (g) 7 days, (h) 9 days, (i) 4 days, (j) 5 days, (k) 7 days, (l) 9 days. Arrows mark duplicated wing pouches.

BAKER 1988). $l(2)ft^{fd}$ $l(2)gd^{1}$ double homozygotes do not show expansion of Wg expression in the leg discs even though leg discs from $l(2)gd^{1}$ homozygotes do (BURATOVICH and BRYANT 1995). In wing discs from $l(2)ft^{fd}$ $l(2)gd^{1}$ larvae, Wg expression covers the overgrowing wing pouch, in a pattern similar to that observed in 4-day wing discs from wild-type larvae (Figure 5, e–h; PHILLIPS and WHITTLE 1993; WILLIAMS *et al.* 1993), and

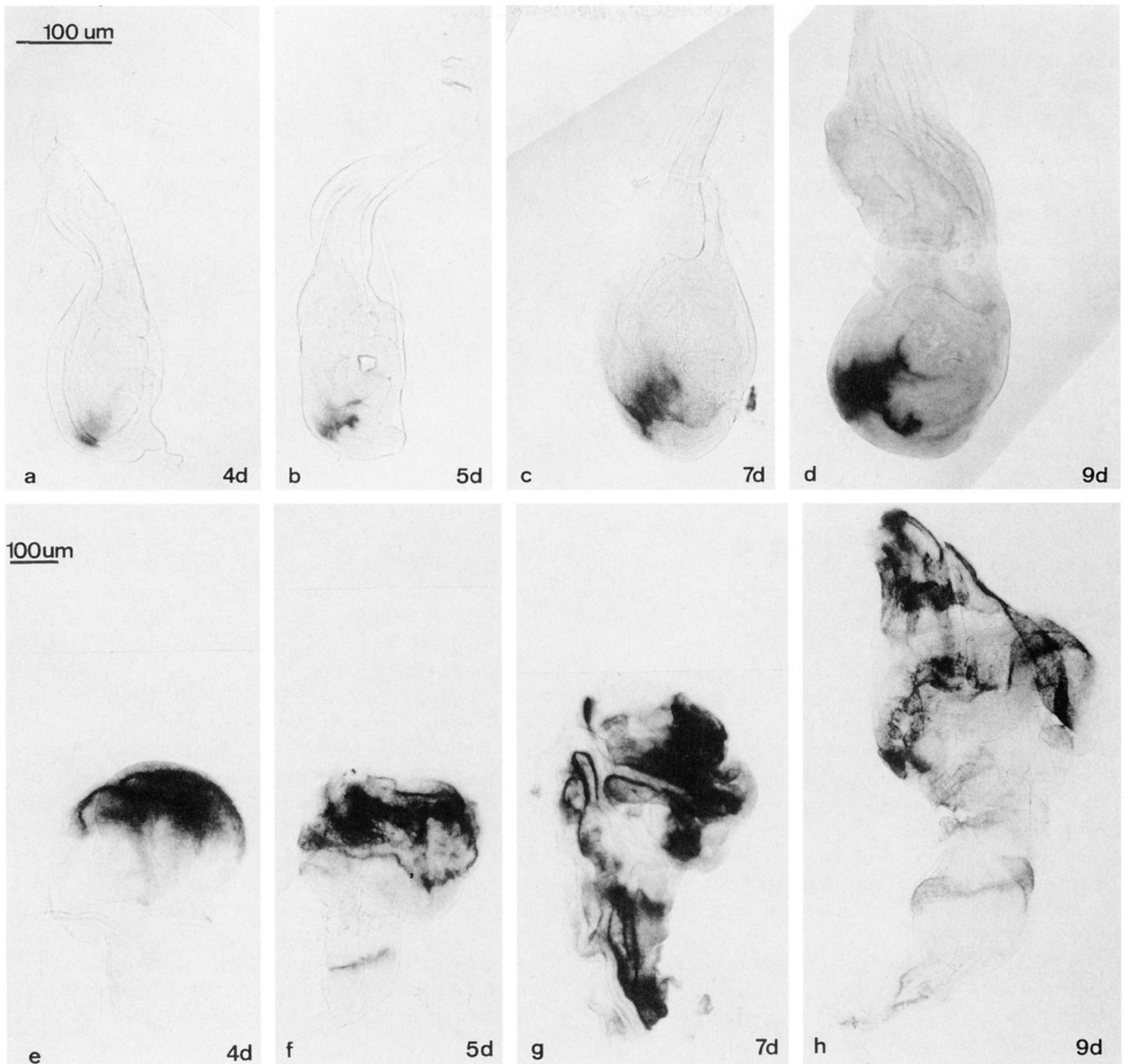

FIGURE 5.—*In situ* hybridization with Wg mRNA to third-leg (a–d) and wing (e–h) discs from *y; l(2)ft^fd dp^ovn l(2)gd^1 a px or* larvae. Discs are shown as follows: (a) 4 days, (b) 5 days, (c) 7 days, (d) 9 days, (e) 4 days, (f) 5 days, (g) 7 days, (h) 9 days.

never completely resolves to the wild-type pattern (BAKER 1988). Therefore, *l(2)ft^fd l(2)gd^1* imaginal discs show alterations of Wg expression that are different from those seen in *l(2)gd^1* discs.

G l(2)gd^1/l(2)gd^1 discs resemble l(2)ft^fd l(2)gd^1 in their pattern of Wg expression: The Wg expression pattern is normal in leg discs from 5 to 9 days *G l(2)gd^1/wgNZ l(2)gd* larvae, as shown by staining with X-Gal to detect expression of the Wg enhancer trap (data not shown). Wing discs from *G l(2)gd/l(2)gd* larvae show Wg expression throughout the wing pouch, which never resolves to a wild-type pattern (data not shown). Therefore discs from *G l(2)gd^1/l(2)gd^1* larvae show a pattern of Wg expression that resembles that of *l(2)ft l(2)gd.*

***G l(2)gd^1/l(2)gd^1* discs show altered Dpp expression:** Dpp expression is greatly expanded in 9-day *G l(2)gd^1/l(2)gd^1 BS3.0* leg discs (Figure 6, f–j) relative to either wild-type (Figure 7a) or *l(2)gd^1* leg discs (Figure 6f), as shown by staining for X-Gal to detect Dpp expression from the *BS3.0* transgene (BLACKMAN *et al.* 1991). Dpp expression also apparently extends into the posterior region of the disc in *G l(2)gd^1/l(2)gd^1*, even though neither wild-type nor *l(2)gd^1* discs ever show Dpp expression in this region (MASUCCI *et al.* 1990; BLACKMAN *et al.* 1991; BURATOVICH and BRYANT 1995). Wing discs also show a large field of ectopic Dpp expression, apparently in the posterior compartment of the wing disc (data not shown). Thus *G*, in only one copy,

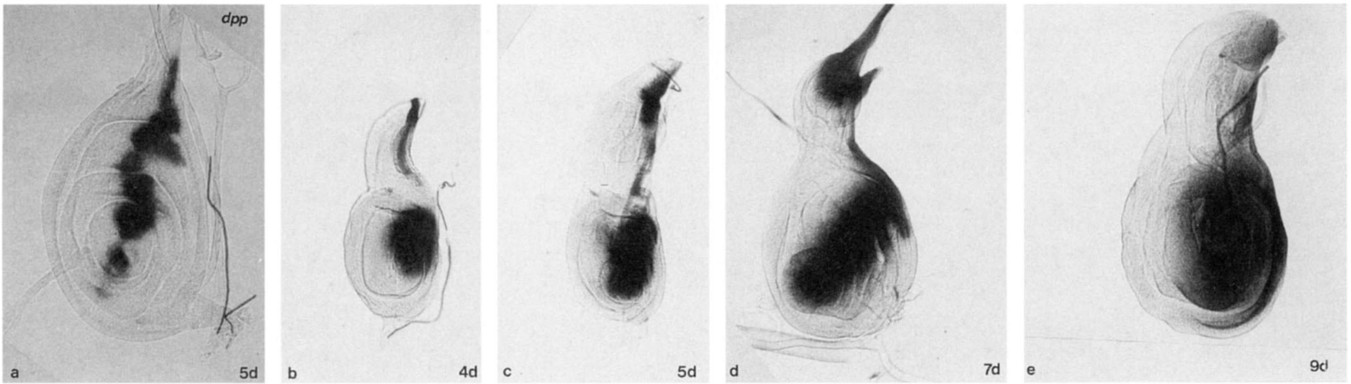

FIGURE 6.—Third leg discs stained with X-Gal to visualize Dpp expression. a is from a *l(2)gd*^{1} *BS3.0/BcGla* larva, and b–e are from *al G l(2)gd*^{1} *a px or/l(2)gd*^{1} *BS3.0* larvae. Discs are shown as follows: (a) 5 days, (b) 4 days, (c) 5 days, (d) 7 days, (e) 9 days.

modifies patterns of Dpp expression in a *l(2)gd*^{1} background.

The enhancement of *l(2)gd* by *l(2)ft* is mediated by Dpp: Third-leg discs from *l(2)gd*^{1} larvae show an extension of the normal stripe of Dpp expression that appears to be necessary for disc duplication (BURATOVICH and BRYANT 1995) and is not seen in the non-duplicating second-leg discs (compare Figure 7, a–b with Figure 7c). However, a stronger allele of *l(2)gd* (*l(2)gd*^{d7}) shows both duplication and extension of the stripe of Dpp expression in the second leg (data not shown). Similarly, 9d *l(2)ft*^{fd} *l(2)gd*^{1}/*l(2)gd*^{1} *BS3.0* second leg discs show ectopic Dpp expression similar to that seen in the third leg discs and they show duplication of the end knob (Figure 7, c and d). The eye-antenna disc also shows ectopic Dpp expression at the site of the second antenna knob, which is not seen in *l(2)gd*^{1} or wild type (Figure 7, e–g). Thus there is a good correlation between duplicate formation and ectopic Dpp expression in these genotypes.

dpp^{d5} is a class II allele of *dpp* (SPENCER *et al.* 1982) that contains a small deletion in the 3′ disc transcriptional enhancer, but leaves the coding region intact (ST. JOHNSTON *et al.* 1990). *dpp*^{d5} homozygotes show no leg defects, and Dpp expression is unaffected in the leg disc (ST. JOHNSTON *et al.* 1990; BLACKMAN *et al.* 1991), but the wing is greatly reduced (SPENCER *et al.* 1982; MASUCCI *et al.* 1990), due to extensive cell death in the wing pouch (BRYANT 1988). *dpp*^{d5} prevents both ectopic Dpp expression and disc duplication in *l(2)gd*^{1} larvae (BURATOVICH and BRYANT 1995). To more precisely determine the role of Dpp in enhancing the duplicative ability of second leg discs in *l(2)ft*^{fd} *l(2)gd*^{1}/ *l(2)gd*^{1} larvae, we added the *dpp*^{d5} mutation to the previously mentioned genotype. No duplicated leg discs from 9-day *dpp*^{d5} *l(2)ft*^{fd} *l(2)gd*^{1}/*dpp*^{d5} *l(2)gd*^{1} *BS3.0* (including second and third leg discs) were observed ($N = 43$; data not shown). These discs also did not show ectopic Dpp expression (data not shown). This demonstrates that ectopic Dpp expression is associated with and required for second-leg disc duplication in *l(2)ft*^{fd} *l(2)gd*^{1}/*l(2)gd*^{1} larvae, and we therefore conclude that enhancement of second-leg disc duplication by *l(2)ft*^{fd} is mediated by Dpp.

ds^{38k} causes enhancement of Dpp expression in *l(2)gd* similar to that of *l(2)ft*: Imaginal discs from *ds*^{38k} *l(2)gd*^{1}/ *l(2)gd*^{1} larvae were studied to determine the effect of one copy of *ds*^{38k} on the disc phenotype of *l(2)gd*^{1}. Imaginal discs from *ds*^{38k} *l(2)gd*^{1}/*l(2)gd*^{1} larvae resemble those of *l(2)ft*^{fd} *l(2)gd*^{1}/*l(2)gd*^{1} rather than *l(2)gd*^{1}, in that the wing, haltere, second and third leg discs are duplicated (data not shown; Table 1). Thus *ds*^{38k} enhances the phenotype of *l(2)gd*^{1} in either one or two copies, as does *l(2)ft*^{fd}. Duplication of the eye-antenna disc in *l(2)gd*^{1}/ *ds*^{38k} *l(2)gd*^{1} larvae also correlates with ectopic Dpp expression at the site of duplicate formation (data not shown), just as it does in *l(2)ft*^{fd} *l(2)gd*^{1}/*l(2)gd*^{1}. Wing discs from *l(2)gd*^{1} *BS3.0/ds*^{38k} *l(2)gd*^{1} larvae apparently show a large area of Dpp expression in the posterior portion of the disc, which enlarges as the disc grows (Figure 8, a–d). This region of increased Dpp expression is also the site of future disc outgrowths. Thus, in one or two copies, *ds*^{38k} not only exaggerates overgrowth and duplication frequency, but also alters Dpp expression in a spatially correlated fashion in *l(2)gd*^{1} imaginal discs.

l(2)gd^{1} *cg*^{1} *discs show extensions of Dpp expression:* Discs from *l(2)gd*^{1} *cg*^{1}*; BS3.0* larvae stained with X-gal show extensions of Dpp expression similar to those seen in *l(2)ft*^{fd} *l(2)gd*^{1}/*l(2)gd*^{1} discs (data not shown). Therefore, *cg*^{1} is also an enhancer of *l(2)gd* and operates by enhancing the ectopic expression of Dpp.

DISCUSSION

We have shown that the addition of homozygous *l(2)ft*, *ds*, and *cg* mutations can greatly increase the amount and rate of overgrowth of *l(2)gd* imaginal discs, while the addition of heterozygous *l(2)ft*, *ds*, or homozygous *cg* mutations can enhance the frequency of duplication of *l(2)gd* imaginal discs. This enhancement of hyperplasia is concurrent with an alteration in the expression of Wg and Dpp, two genes that play a central role in imaginal disc patterning (LAWRENCE and STRUHL 1996). Also,

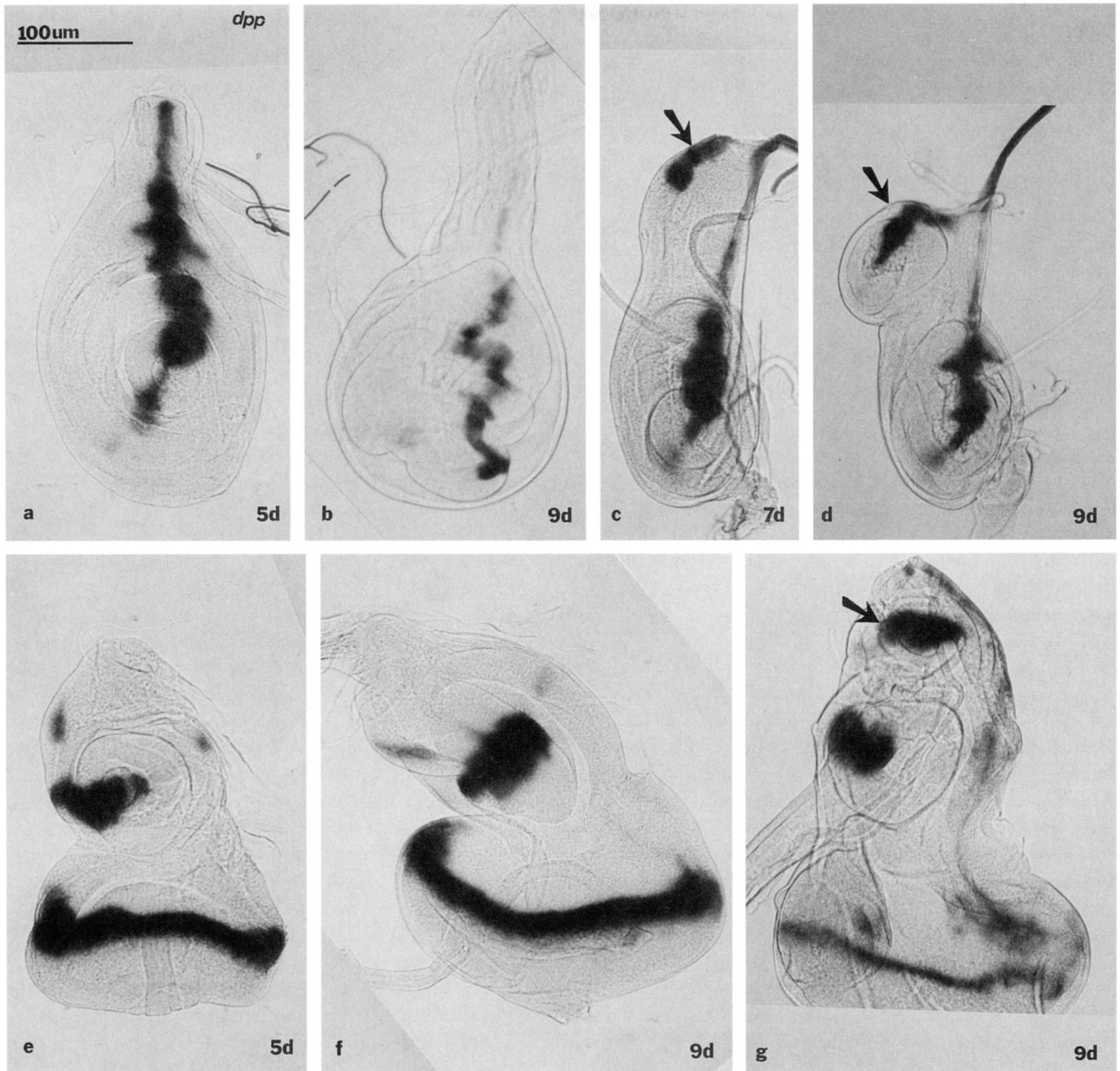

FIGURE 7.—Imaginal discs stained with X-Gal to visualize Dpp expression. (a) Second leg disc from *l(2)gd[1] BS3.0/CyO* larva at 5 days. (b) Second leg disc from *l(2)gd[1] BS3.0* larva at 9 days. (c) Second leg disc from a *l(2)ft[fd] l(2)gd[1]/l(2)gd[1] BS3.0* larva at 7 days. Ectopic Dpp expression is marked with an arrow. (d) Second leg disc from a *l(2)ft[fd] l(2)gd[1]/l(2)gd[1] BS3.0* larva at 9 days. Duplicate end knob is marked with an arrow. (e) Eye-antenna disc from a *l(2)gd[1] BS3.0/CyO* larva at 5 days. (f) Eye-antenna disc from a *l(2)gd[1] BS3.0* larva at 9 days. (g) Eye-antenna disc from a *l(2)ft[fd] l(2)gd[1]/l(2)gd[1] BS3.0* larva at 9 days. Duplicate antenna knob is marked with an arrow.

l(2)gd mitotic clones do not overgrow, but when the *l(2)gd* mutation is combined with mutations in certain other genes the mitotic clones protrude from surrounding normal tissue, suggesting excessive growth and increased growth rates within the clones.

One of the mutations that enhances the hyperplastic phenotype of *l(2)gd* is *l(2)ft*, which even when heterozygous increases the propensity of discs from *l(2)gd* larvae to duplicate. The *l(2)ft* gene encodes a giant cadherin-like molecule (MAHONEY *et al.* 1991) whose role in cell adhesion is unclear, but pharate *l(2)ft* adults and mitotic clones of *l(2)ft* show separated vesicles of cuticle, indicating a failure of cell adhesion (BRYANT *et al.* 1988). *Gull,* the dominant allele of *l(2)ft,* causes dramatic exaggeration of overgrowth in *l(2)gd* discs. Given the potential role of Fat in cell adhesion, the enhancement of overgrowth and imaginal disc duplication in *l(2)gd* by *l(2)ft* is probably due to the mechanisms of cell proliferation control being very sensitive to perturbations in cell adhesion. Because cell adhesion and signaling are

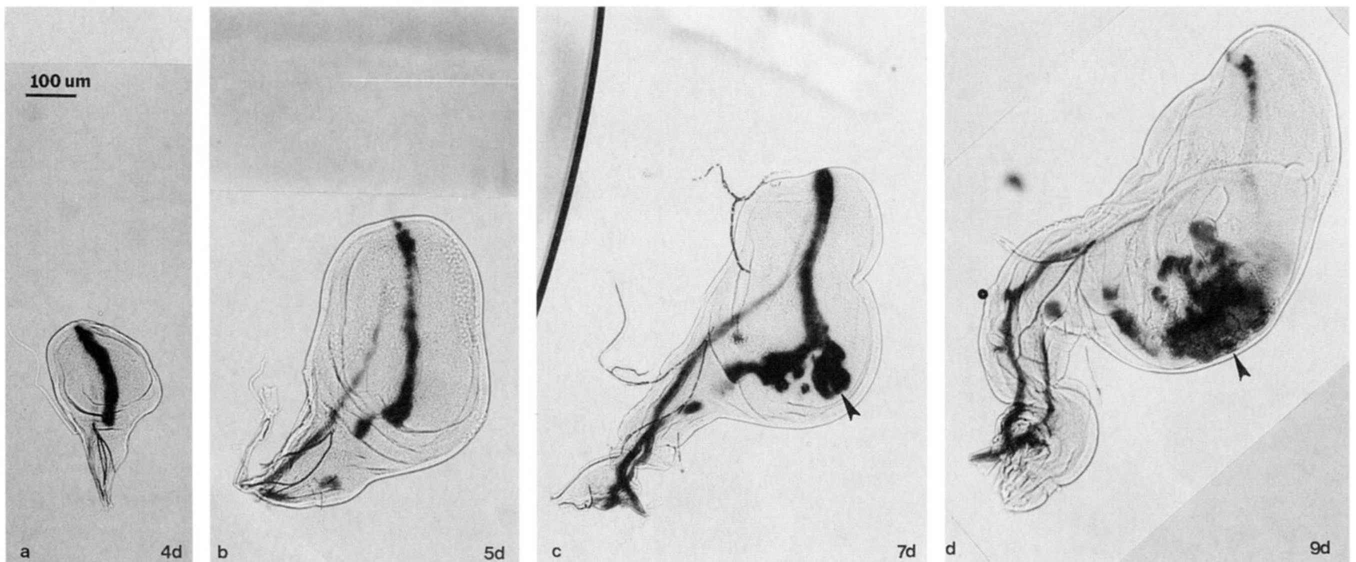

FIGURE 8.—Wing (a–d) imaginal discs from *ds*^{38k} *l(2)gd*^{1} *or/l(2)gd*^{1} *BS3.0* larvae stained with X-Gal to visualize Dpp expression. Discs shown as follows: (a) 4 days, (b) 5 days, (c) 7 days (arrow marks ectopic Dpp expression), (d) 9 days (arrow marks ectopic Dpp expression).

closely related functions in epithelial cells (WOODS and BRYANT 1993), disruptions of adhesion probably interfere with cell signaling.

The enhancement of *l(2)gd* imaginal disc overgrowth by *ds* supports the conclusion that cell proliferation control is affected by subtle disruptions in cell adhesion. *ds* was chosen for these studies because it, like *l(2)ft,* has been shown to encode a member of the cadherin family of calcium-dependent cell adhesion molecules, although it lacks the EGF-like and laminin A G-domain-like repeats of the Fat protein (CLARK *et al.* 1995). It also interacts with *l(2)ft,* as shown by that fact that *ds* mutations act as enhancers of the viable alleles and suppressors of the dominant allele *Gull* (MOHR 1929; CLARK *et al.* 1995). *ds* homozygotes show broad wings, malformed nota and shortened, swollen tarsi, (WADDINGTON 1943; WADDINGTON 1953), but no obvious defects in imaginal discs. CLARK *et al.* (1995) have postulated that the extracellular domains of the Ds and Fat proteins form both homo- and hetero-dimers that act to control either cell proliferation or morphogenesis. According to this model, the *Gull* mutation could produce a dominant phenotype by strengthening the interaction between Fat and Ds, leading to decreased Ds homodimer formation and consequent abnormalities in morphogenesis. The enhancement of imaginal disc overgrowth in *l(2)gd* by *ds* is, possibly, another example of disruption of cell adhesion leading to breakdown in cell-cell signaling. *ds* and *l(2)ft* could both interfere with signaling by causing subtle disruptions of cell adhesion. The human DCC gene, which encodes a large molecule that shows homology to neural cell adhesion molecule (FEARON *et al.* 1990; HEDRICK *et al.* 1992) and might be the receptor for the axonal chemoattractant netrin-1 (KEINO-MASU *et al.* 1996; FAZELI *et al.* 1997), shows frequent loss of heterozygosity in colorectal adenocarcinomas, prostatic carcinoma, esophageal cancers, and hematologic malignancies (CHO and VOGELSTEIN 1992; HUANG *et al.* 1992; GAO *et al.* 1993; PORFIRI *et al.* 1993), providing another possible example of how disruption of cell adhesion can promote the tumorous phenotype.

cg mutations interact with *l(2)gd* in modifying the amount of overgrowth as well as the frequency of imaginal disc duplication. *ds* exaggerates the phenotype of *cg,* and of two other mutations that do not themselves cause overgrowth: *four jointed* (*fj*), and *dachs* (*d*) (WADDINGTON 1943; 1953). *cg* mutants have very large sex combs on the legs of the male, the females are completely sterile, and wing vein L4 has a gap (WADDINGTON 1953). *cg* interacts with *ds* in that the double homozygote shows legs that are quite shortened and swollen, the nota have twisted malformations, and the wings are very short and reduced (WADDINGTON 1943). WADDINGTON (1943) concluded from this that *ds* acts as an exaggerator of *cg* phenotypes, but he also postulated that the cuticular defects in *ds cg* double homozygotes were due to a combination of extra tissue and abnormal eversion. Therefore, *ds*$^{+}$, *cg*$^{+}$, and the genes with which they interact might control cell proliferation locally. The highly localized expression and mutant phenotypes of the four-jointed gene, and the suppression of those phenotypes by *Gull* or *expanded* alleles is consistent with this hypothesis (VILLANO and KATZ 1995; BRODSKY and STELLER 1996). The enhancement of overgrowth in *l(2)gd* by *cg* and *ds* might then be due to local enhancement of cell proliferation, a possibility that is supported by the phenotypes of the double homozygotes.

Hyperplastic imaginal discs often show misregulation of genes that are important in the establishment of pattern (this study; BURATOVICH and BRYANT 1995). Neoplastic discs from *l(2)gl* larvae also show multiple

patches of ectopic *engrailed* and *wingless* expression (AGRAWAL *et al.* 1995; MUKHERJEE *et al.* 1995). In addition, the exaggeration of hyperplasia by the addition of overgrowth or patterning mutations is associated with further abnormalitites in gene expression. Some gene misexpression is probably a consequence of hyperplasia. But in other cases, gene misexpression might actually directly contribute to the exaggeration of hyperplasia. Wg and Dpp are ectopically expressed in many of the hyperplastic discs examined in this study. Experimentally induced ectopic expression of Wg (CAMPBELL *et al.* 1993; STRUHL and BASLER 1993; COUSO *et al.* 1995; DIAZ-BENJUMEA and COHEN 1995; WILDER and PERRIMON 1995; NEUMANN and COHEN 1996, 1997; NG *et al.* 1996; ZECCA *et al.* 1997) and Dpp (CAPDEVILA and GUERRERO 1994; DIAZ-BENJUMEA *et al.* 1994; ZECCA *et al.* 1995; LECUIT *et al.* 1996; MORIMURA *et al.* 1996; NELLEN *et al.* 1996) can cause both local and long-range stimulation of cell proliferation, and might do so in imaginal discs from many of the genotypes examined in this work. In discs from *G l(2)gd/l(2)gd* larvae, for example, Dpp expression is abnormal from the beginning of the third larval instar, and Dpp expression also apparently occurs in the cells of the posterior compartment. Because Dpp is a molecule that causes such extensive effects on cell proliferation, it is likely that some of the exaggeration of hyperplasia observed in *G l(2)gd/l(2)gd* discs is due to the ectopic expression of Dpp.

Hyperplasia, then, can be influenced by mutations in combinations of tumor suppressor genes and by overall changes in gene expression of certain regulatory gene products, such as Wg or Dpp-like molecules. We suggest that various changes in patterned gene expression can sometimes accompany hyperplasia, and under some circumstances, play a role in causing excess growth.

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LITERATURE CITED

AGRAWAL, N., M. KANGO, A. MISHRA and P. SINHA, 1995 Neoplastic transformation and aberrant cell-cell interactions in genetic mosaics of *lethal(2)giant larvae lgl*, a tumor suppressor gene of *Drosophila*. Dev. Biol. **172:** 218–229.

BAKER, N. E., 1988 Transcription of the segment-polarity gene *wingless* in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal *wg* mutation. Development **102:** 489–497.

BERNS, A., N. VAN DER LUGT, M. ALKEMA, M. VAN LOHUIZEN, J. DOMEN *et al.*, 1994 Mouse model systems to study multistep tumorigenesis. Cold Spring Harbor Symp. Quant. Biol. **59:** 435–447.

BLACKMAN, R. K., M. SANICOLA, L. A. RAFTERY, T. GILLEVET and W. M. GELBART, 1991 An extensive 3′ *cis*-regulatory region directs the imaginal disk expression of *decapentaplegic*, a member of the TGF-β family in *Drosophila*. Development **111:** 657–666.

BRODSKY, M. H., and H. STELLER, 1996 Positional information along the dorsal-ventral axis of the *Drosophila* eye: graded expression of the *four-jointed* gene. Dev. Biol. **173:** 428–446.

BRYANT, P. J., 1988 Localized cell death caused by mutations in a *Drosophila* gene coding for a transforming growth factor-beta homolog. Dev Biol. **128:** 386–395.

BRYANT, P. J., and P. LEVINSON, 1985 Intrinsic growth control in the imaginal primordia of *Drosophila*, and the autonomous action of a lethal mutation causing overgrowth. Dev. Biol. **107:** 355–363.

BRYANT, P. J., and G. SCHUBIGER, 1971 Giant and duplicated imaginal discs in a new lethal mutant of *Drosophila melanogaster*. Dev. Biol. **24:** 233–263.

BRYANT, P. J., B. HUETTNER, L. I. HELD, JR., J. RYERSE and J. SZIDONYA, 1988 Mutations at the *l(2)ft* locus interfere with cell proliferation control and epithelial morphogenesis in *Drosophila*. Dev. Biol. **129:** 541–554.

BURATOVICH, M. A., and P. J. BRYANT, 1995 Duplication of *l(2)gd* imaginal discs in *Drosophila* is mediated by ectopic expression of *wg* and *dpp*. Dev. Biol. **168:** 452–463.

CAMPBELL, G., T. WEAVER and A. TOMLINSON, 1993 Axis specification in the developing *Drosophila* appendage: the role of *wingless*, *decapentaplegic*, and the homeobox gene *aristaless*. Cell **74:** 1113–1123.

CAPDEVILA, J., and I. GUERRERO, 1994 Targeted expression of the signaling molecule decapentaplegic induces pattern duplications and growth alterations in *Drosophila* wings. EMBO J. **13:** 4459–4468.

CHAN, L-N., and W. GEHRING, 1971 Determination of blastoderm cells in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **68:** 2217–2221.

CHO, K. R., and B. VOGELSTEIN, 1992 Suppressor gene alterations in the colorectal adenoma-carcinoma sequence. J. Cell. Biochem. **50** (Suppl.) **16G:** 137–141.

CLARK, H. F., D. BRENTRUP, K. SCHNEITZ, A. BIEBER, C. GOODMAN *et al.*, 1995 *Dachsous* encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*. Genes Dev. **9:** 1530–1542.

COUSO, J. P., E. KNUST and A. MARTÍNEZ-ARIAS, 1995 *Serrate* and *wingless* cooperate to induce *vestigial* gene expression and wing formation in *Drosophila*. Curr. Biol. **5:** 1437–1448.

DIAZ-BENJUMEA, F. J., B. COHEN and S. M. COHEN, 1994 Cell interaction between compartments establishes the proximal-distal axis of *Drosophila* legs. Nature **372:** 175–179.

DIAZ-BENJUMEA, F. J., and S. M. COHEN, 1993 Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. Cell **75:** 741–752.

DIAZ-BENJUMEA, F. J., and S. M. COHEN, 1995 Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. Development **121:** 4215–4225.

FAZELI, A., S. L. DICKINSON, M. L. HERMISTON, R. V. TIGHE, R. G. STEEN *et al.*, 1997 Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. Nature **386:** 796–804.

FEARON, E. R., K. R. CHO, J. M. NIGRO, S. E. KERN, J. W. SIMONS *et al.*, 1990 Identification of a chromosome 18q gene that is altered in colorectal cancers. Science **247:** 49–56.

GAO, X., K. V. HONN, D. GRIGNON, W. SAKR and Y. Q. CHEN, 1993 Frequent loss of expression and loss of heterozygosity of the putative tumor suppressor gene *DCC* in prostatic carcinomas. Cancer Res. **53:** 2723–2727.

GATEFF, E., 1978 Malignant neoplasms of genetic origin in *Drosophila melanogaster*. Science **200:** 1448–1459.

GATEFF, E., 1994 Tumor suppressor and overgrowth suppressor genes of *Drosophila melanogaster*. Developmental aspects. Int. J. Dev. Biol. **38:** 565–590.

GAZDAR, A. F., 1994 The molecular and cellular basis of human lung cancer. Anticancer Res. **14:** 261–267.

HARTENSTEIN, V., and J. W. POSAKONY, 1989 Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development **107:** 389–405.

HEDRICK, L., K. R. CHO, J. BOYD, J. RISINGER and B. VOGELSTEIN, 1992 *DCC*: a tumor suppressor gene expressed on the cell surface. Cold Spring Harbor Symp. Quant. Biol. **57:** 345–352.

HUANG, F., C. DAMBLY-CHAUDIÉRE and A. GHYSEN, 1991 The emergence of sense organs in the wing disc of *Drosophila*. Development **111:** 1087–1095.

HUANG, Y., R. F. BOYNTON, P. L. BLOUNT, R. J. SILVERSTEIN, J. YIN *et al.*, 1992 Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. Cancer Res. **52:** 6525–6530.

KEINO-MASU, K., M. MASU, L. HINCK, E. D. LEONARDO, S. S. Y. CHAN

et al., 1996 Deleted in colon cancer (DCC) encodes a netrin receptor. Cell **87:** 175–185.

KLEIN-SZANTO, A. J. P., 1991 The role of chemically induced epithelial hyperplasia in the development of human cancer. Prog. Clin. Biol. Res. **369:** 35–41.

LAWRENCE, P. A., and G. STRUHL, 1996 Morphogens, compartments, and pattern: lessons from *Drosophila*? Cell **85:** 951–961.

LECUIT, T., W. J. BROOK, M. NG, M. CALLEJA, H. SUN *et al.*, 1996 Two distinct mechanisms for long-range patterning by Decapentaplegic in the *Drosophila* wing. Nature **381:** 387–393.

LEVINE, A. C., 1995 Pathogenesis and medical management of benign prostatic hyperplasia. Trends Endocrinol. Metab. **6:** 128–132.

MAHONEY, P. A., U. WEBER, P. ONOFRECHUK, H. BIESSMANN, P. J. BRYANT *et al.*, 1991 The *l(2)ft* tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. Cell **67:** 853–868.

MASUCCI, J. D., R. J. MILTENBERGER and F. M. HOFFMANN, 1990 Pattern-specific expression of the *Drosophila decapentaplegic* gene in imaginal disks is regulated by 3′ *cis*-regulatory elements. Genes Dev. **4:** 2011–2023.

MCDONNELL, T. J., and S. J. KORSMEYER, 1991 Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). Nature **349:** 254–256.

MECKE, H., J. LUTTGES, E. LEHMANN-WILLENBROOCK, P. KUNSTMANN and I. FREYS, 1994 Treatment of precursors of endometrial cancer. Gynecol. Obstet. Invest. **37:** 130–134.

MIRSALIS, J. C., and K. L. STEINMETZ, 1990 The role of hyperplasia in liver carcinogenesis. Prog. Clin. Biol. Res. **331:** 149–162.

MOHR, O., 1929 Exaggeration and inhibition phenomena encountered in the analysis of an autosomal dominant. Z. Induk. Abstamm. Vererbungs. **50:** 113–200.

MORATA, G., and P. RIPOLL, 1975 Minutes: mutants of *Drosophila* autonomously affecting cell division rate. Dev. Biol. **42:** 211–221.

MORGAN-PARKES, J. H., 1995 Metastases: mechanisms, pathways, and cascades. AJR. Am. J. Roentgenol. **164:** 1075–1082.

MORIMURA, S., L. MAVES, Y. CHEN and F. M. HOFFMANN, 1996 *decapentaplegic* overexpression affects *Drosophila* wing and leg imaginal disc development and *wingless* expression. Dev. Biol. **177:** 136–151.

MUKHERJEE, A., S. C. LAKHOTIA and J. K. ROY, 1995 *l(2)gl* gene regulates late expression of segment polarity genes in *Drosophila*. Mech. Dev. **51:** 227–234.

NELLEN, D., R. BURKE, G. STRUHL and K. BASLER 1996 Direct and long-range action of a Dpp morphogen gradient. Cell **85**: 357–368.

NEUMANN, C. J., and S. M. COHEN, 1996 Distinct mitogenic and cell fate specification functions of *wingless* in different regions of the wing. Development **122:** 1781–1789.

NEUMANN, C. J., and S. M. COHEN, 1997 Long-range action of Wingless organizes the dorsal-ventral axis of the *Drosophila* wing. Development **124:** 871–880.

NG, M., F. J. DIAZ-BENJUMEA, J. P. VINCENT, J. WU and S. M. COHEN, 1996 Specification of the wing by localized expression of wingless protein. Nature **381:** 316–318.

PHILLIPS, R. G., I. J. H. ROBERTS, P. W. INGHAM and J. R. S. WHITTLE, 1990 The *Drosophila* segment polarity gene *patched* is involved in a position-signalling mechanism in imaginal discs. Development **110:** 105–114.

PHILLIPS, R. G., and J. R. S. WHITTLE, 1993 *wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. Development **118:** 427–438.

PORFIRI, E., L. M. SECKER-WALKER, A. V. HOFFBRAND and J. F. HANCOCK, 1993 *DCC* tumor suppressor gene is inactivated in hematologic malignancies showing monosomy 18. Blood **81:** 2696–2701.

SIMPSON, P., and G. MORATA, 1981 Growth and cell competition in *Drosophila*. J. Embryol. Exp. Morphol. **65:** 77–88.

SPENCER, F. A., F. M. HOFFMANN and W. M. GELBART, 1982 Decapentaplegic: a gene complex affecting morphogenesis in *Drosophila melanogaster*. Cell **28:** 451–461.

ST. JOHNSTON, R. D., F. M. HOFFMANN, R. K. BLACKMAN, D. SEGAL, R. GRIMAILA *et al.*, 1990 Molecular organization of the *decapentaplegic* gene in *Drosophila melanogaster*. Genes Dev. **4:** 1114–1127.

STRUHL, G., and K. BASLER, 1993 Organizing activity of wingless protein in *Drosophila*. Cell **72:** 527–540.

TAN, A. K., T. L. TEWFIK, B. MOROZ, M. J. THIBAULT and K. WATTERS, 1995 Focal epithelial hyperplasia. Otolaryngol. Head. Neck Surg. **112:** 316–320.

TIMMONS, L., E. HERSPERGER, E. WOODHOUSE, J. XU, L-Z. LIU *et al.*, 1993 The expression of the *Drosophila awd* gene during normal development and in neoplastic brain tumors caused by *lgl* mutations. Dev. Biol. **158:** 364–379.

USUI, K., and K. KIMURA, 1993 Sequential emergence of the evenly spaced microchaetes on the notum of *Drosophila*. Roux's Arch. Dev. Biol. **203:** 151–158.

VILLANO, J. L., and F. N. KATZ, 1995 *four-jointed* is required for intermediate growth in the proximal-distal axis in *Drosophila*. Development **121:** 2767–2777.

VOGELSTEIN, B., and K. W. KINZLER, 1993 The multistep nature of cancer. Trends Genet. **4:** 138–141.

WADDINGTON, C. H., 1943 The development of some 'leg genes' in *Drosophila*. J. Genet. **43:** 29–45.

WADDINGTON, C. H., 1953 The interactions of some morphogenetic genes in *Drosophila melanogaster*. J. Genet. **51:** 243–258.

WATSON, K. L., R. W. JUSTICE and P. J. BRYANT, 1994 *Drosophila* in cancer research: the first fifty tumor suppressor genes. J. Cell Sci. **107** (Suppl.) **18:** 19–33.

WILDER, E. L., and N. PERRIMON, 1995 Dual functions of *wingless* in the *Drosophila* leg imaginal disc. Development **121:** 477–488.

WILLIAMS, J. A., S. W. PADDOCK and S. B. CARROLL, 1993 Pattern formation in a secondary field—a hierarchy of regulatory genes subdivides the developing *Drosophila* wing into discrete subregions. Development **117:** 571–584.

WOODS, D. F., and P. J. BRYANT, 1993 Apical junctions and cell signaling in epithelia. J. Cell Sci. Suppl. **17:** 171–181.

ZECCA, M., K. BASLER and G. STRUHL, 1995 Sequential organizing activities of *engrailed, hedgehog* and *decapentaplegic* in the *Drosophila* wing. Development **121:** 2265–2278.

ZECCA, M., K. BASLER and G. STRUHL, 1996 Direct and long-range action of a Wingless morphogen gradient. Cell **87:** 833–844.

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