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Interactions of *decapentaplegic*, *wingless*, and *Distal-less* in the *Drosophila* leg

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Abstract. The genes *decapentaplegic*, *wingless*, and *Distal-less* appear to be instrumental in constructing the anatomy of the adult *Drosophila* leg. In order to investigate how these genes function and whether they act coordinately, we analyzed the leg phenotypes of the single mutants and their *inter se* double mutant compounds. In *decapentaplegic* the tarsi frequently exhibit dorsal deficiencies which suggest that the focus of gene action may reside dorsally rather than distally. In *wingless* the tarsal hinges are typically duplicated along with other dorsal structures, confirming that the hinges arise dorsally. The plane of symmetry in double-ventral duplications caused by *decapentaplegic* is virtually the same as the plane in double-dorsal duplications caused by *wingless*. It divides the fate map into two parts, each bisected by the dorsoventral axis. In the double mutant *decapentaplegic wingless* the most ventral and dorsal tarsal structures are missing, consistent with the notion that both gene products function as morphogens. In *wingless Distal-less* compounds the legs are severely truncated, indicating an important interaction between these genes. *Distal-less* and *decapentaplegic* manifest a relatively mild synergism when combined.

Key words: Positional information– Pattern formation – Developmental genetics – Polar Coordinate Model – Janus mutants – bristles

Introduction

The six legs of a *Drosophila* adult originate as clusters of cells on the flank of the embryo (Bate and Martinez Arias 1991; Cohen et al. 1991). The clusters invaginate to form hollow sacs – the imaginal discs – which grow during the larval period and evert during metamorphosis (Gehring and Nöthiger 1973; Fristrom and Fristrom 1975). Two genes appear to pinpoint the sites where the clusters arise: *decapentaplegic* (*dpp*) and *wingless* (*wg*). In 5-h old embryos the leg discs are first detectable where *dpp* and *wg* stripes intersect in each thoracic segment (Cohen et al. 1993): *dpp* is expressed in stripes parallel to the anterior-posterior (AP) axis of the body, while *wg* is expressed in segmentally-repeated stripes parallel to the dorsal-ventral (DV) axis.

These same genes may also designate cellular positions within the leg disc. Thus, *wg* is transcribed in a ventral sector of the disc throughout development (Fig. 1d; Baker 1988; Couso et al. 1993), but ectopic expression on the dorsal side can be artificially induced (Struhl and Basler 1993), leading to a secondary ventral pattern. Duplicated ventral patterns are also found in the tarsi of *dpp* mutants (Spencer et al. 1982; see below). However, unlike *wg*, *dpp* is transcribed in a stripe that spans the disc (Masucci et al. 1990). The *dpp* stripe runs approximately along the DV axis of the third-instar disc and continues to be expressed along the dorsal and ventral midlines of the everting pupal leg (Masucci et al. 1990), though its expression is more intense dorsally than ventrally at both stages. Both the *wg* and *dpp* genes encode secretable growth factors (Gelbart 1989; van den Heuvel et al. 1989; González et al. 1991) which could function as morphogens (Wolpert 1969). In contrast, a third gene which is expressed from the inception of the leg disc (Cohen 1990) – *Distal-less* (*Dll*; a.k.a. *Brista*; Sunkel and Whittle 1987) – contains a homeodomain indicative of a transcription factor (Cohen et al. 1989). In third-instar discs *Dll* is expressed in a central region including the tarsus and distal tibia, plus a separate ring corresponding to the femur and possibly trochanter (Cohen 1993). *Dll* mutations remove distal leg segments, and stronger alleles remove more segments (Cohen and Jürgens 1989), suggesting that this gene may encode cellular positions along the proximodistal axis of the leg. Because leg segments arise from concentric rings of cells in the disc (Fristrom and Fristrom 1975), the proximodistal bristle rows (Fig. 1a) correspond to radial spokes in the disc (Fig. 1c). It is not known whether imaginal discs employ a Cartesian (Meinhardt 1983; Gelbart

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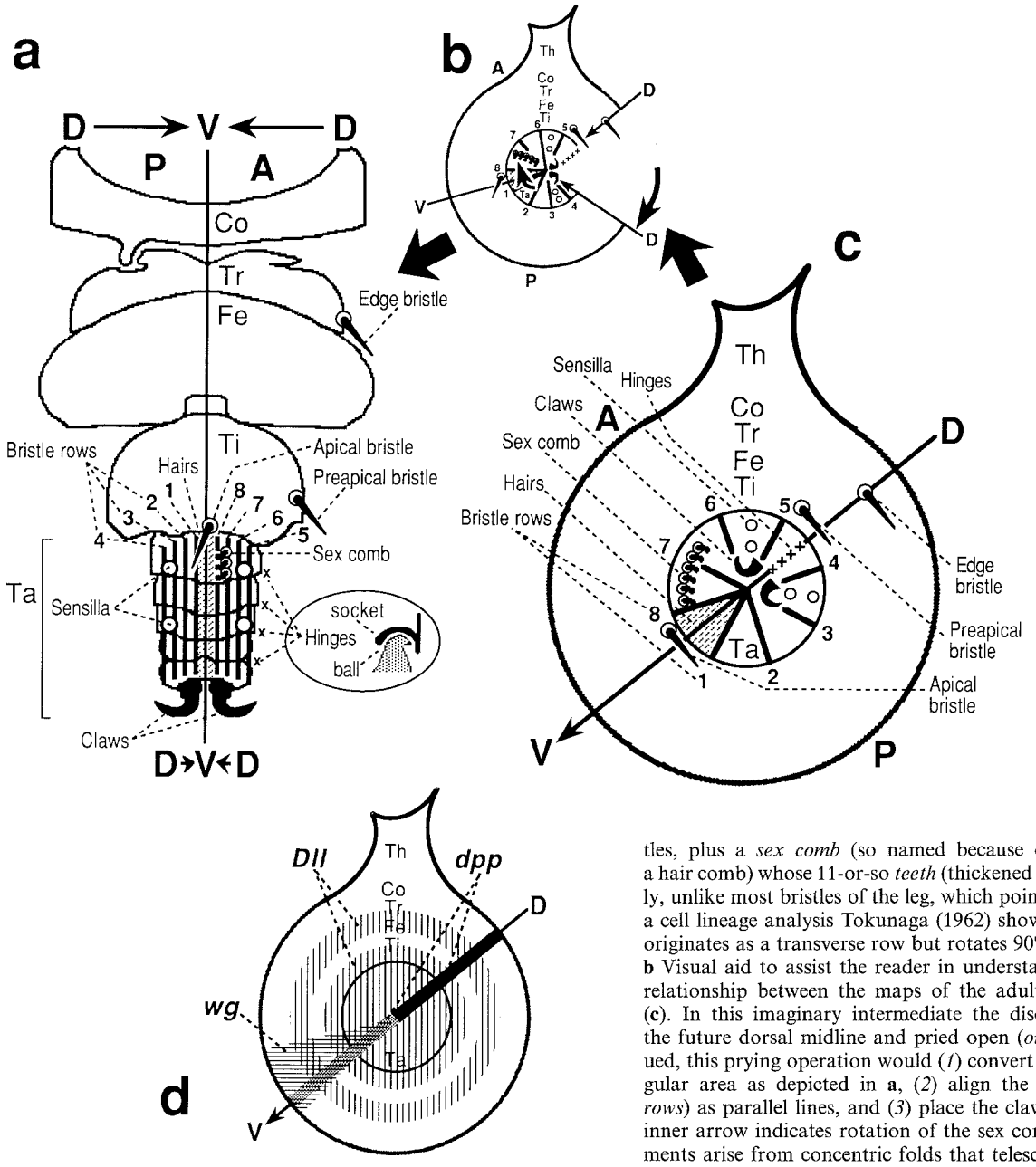

Fig. 1a–d. Maps of cuticular elements of the adult leg, cellular fates within the leg imaginal disc, and intradisc expression domains of the genes *dpp*, *wg*, and *Dll*. **a** Schematic diagram of a left leg of a wild-type fly, with the proximodistal (top-to-bottom) axis foreshortened. The diagram is based upon the nomenclature of Grimshaw (1905), Hannah-Alava (1958), and Schubiger (1968; cf. Bryant 1978). The leg is shown as if it were cut along the dorsal (*D*) midline and laid flat, with the ventral (*V*) midline in the center (*P* = posterior, *A* = anterior). The 9 segments of the leg (from proximal to distal) are the coxa (*Co*), trochanter (*Tr*), femur (*Fe*), tibia (*Ti*), and the 5 tarsal (*Ta*) segments T1 (*the basitarsus*), T2, T3, T4, and T5. The shapes of most segments except the coxa are similar for the three pairs of legs. (The coxal outline here is that of a foreleg.) Convenient markers of the D and V midlines are the trochanteral *edge* bristle and the tibial *apical* and *preapical* bristles (named for their positions at or above the apex of this segment). The latter bristles are distinctive on the second legs where their pigmentation and thickness sets them apart as macrochaetes. The 8 longitudinal rows of bristles on the tarsus are depicted as bold vertical lines. A band of hairs (noninnervated trichomes) lies between rows 1 and 8, two pairs of circular sensilla campaniformia reside dorsally on T1 and T3, and adjoining tarsal segments are hinged (*x's*) at the D midline by ball-and-socket articulations (Held et al. 1986; Held 1990). In place of rows 7 and 8 the male foreleg bears a series (not shown) of transverse (horizontal) rows of bristles, plus a *sex comb* (so named because of its resemblance to a hair comb) whose 11-or-so *teeth* (thickened bristles) point ventrally, unlike most bristles of the leg, which point distally. Based upon a cell lineage analysis Tokunaga (1962) showed that the sex comb originates as a transverse row but rotates 90° during development. **b** Visual aid to assist the reader in understanding the topological relationship between the maps of the adult leg (**a**) and leg disc (**c**). In this imaginary intermediate the disc has been slit along the future dorsal midline and pried open (*outer arrow*). If continued, this prying operation would (*1*) convert the circle into a triangular area as depicted in **a**, (*2*) align the radial spokes (*bristle rows*) as parallel lines, and (*3*) place the claws at the bottom. The inner arrow indicates rotation of the sex comb. In reality the segments arise from concentric folds that telescope out during metamorphosis (Fristrom and Fristrom 1975), converting the circle into a cone, which has then been filleted and flattened to arrive at the map in (**a**). **c** Fate map of the leg imaginal disc. The positions of the segments, sex comb, claws, and edge bristle are based on Schubiger's (1968) map, which was derived from transplantation experiments. Other features are inserted at their presumed sites (see Materials and methods). The cardinal directions (*D*, *V*, *A*, and *P*) refer to future axes of the adult leg. The stalk and peripheral cells form thoracic (*Th*) cuticle that is not part of the leg proper. The disc has a monolayer epithelium (Poodry and Schneiderman 1970). **d** Domains where the genes *dpp* (Masucci et al. 1990), *wg* (Baker 1988), and *Dll* (Cohen 1993) are transcribed. The exact placement of the *dpp* stripe relative to markers in the fate map is unknown: its alignment with the DV axis is inferred from *dpp* expression in the pupal leg where the stripe runs along the dorsal and ventral midlines (Masucci et al. 1990). The *dpp* stripe manifests more intense expression in its dorsal half than in its ventral half (dark *vs.* light shading). The overlap of the *wg* wedge and the *dpp* stripe is deduced from the locations of these areas relative to the *engrailed* sector (not shown; Baker 1988; Masucci et al. 1990; Raftery et al. 1991; Couso et al. 1993; Struhl and Basler 1993). Because of uncertainties in the map, the possibility cannot be excluded that the *wg* sector is actually bisected by the ventral midline. The extent of *Dll* expression in the trochanter is unknown (Cohen 1993)

1989) or polar (French et al. 1976; Wilkins and Gubb 1991; Couso et al. 1993) coordinate system. To investigate how the different axes function in cuticular patterning, we examined leg defects caused by individual *dpp*, *wg*, and *Dll* mutations, and by their double-mutant compounds.

Materials and methods

Fly stocks. The wild-type stock Oregon R was used as a standard for leg anatomy. The three genes analyzed are all on the second chromosome: *dpp* and *wg* are on the left arm at map positions 4.0 and 30.0 respectively, while *Dll* is at the right tip at 107.8. Doubly mutant chromosomes were constructed by genetic recombination from the following starter stocks (cf. Lindsley and Zimm 1992 for markers): (1) dpp^{d6} adh^{fn6} *pr cn/Gla*, (2) dpp^{d12}/*CyO*, (3) wg^{CX3} *b pr/CyO*, (4) wg^{CX4} *b pr/CyO*, (5) Dll^{M}/*SM5*, (6) Dll^{7} *b pr cn* wx^{wxt} *bw/SM6a*, and (7) Dll^{IB}/*CyO*, and confirmed by backcrossing to parental strains. Although some *Dll* mutations have dominant effects on the antenna (as indicated by the capital letter; Sunkel and Whittle 1987), their effects on the leg are recessive, so homozygotes were employed for all studies.

Rearing of flies and mounting of legs. Flies were raised at 25° C on *Drosophila* Instant Medium (Ward's) prepared with a 0.1% aqueous solution of the mold inhibitor Tegosept M, with live yeast added on top of the medium. Adults were not allowed to eclose inside food vials because leg abnormalities often cause sticking to the food. Instead, for every genotype analyzed, pupae were harvested before eclosion, kept in humidified petri dishes, and allowed to develop fully, whereupon the eclosed adults (A) and dead pharate adults (PA) were counted and preserved in 70% ethanol. Following are survival frequencies expressed as numbers of individuals (A:PA), with data for nonmutant siblings (balancer heterozygotes) in same harvested cohort given in parentheses ("*" denotes group from which legs were mounted): dpp^{d6}/dpp^{d12} 79*:69 (598:9), wg^{CX3}/wg^{CX4} 0:151* (557:6), Dll^{M} 99*:0 (323:0), Dll^{7} 236*:82 (702:5), Dll^{IB} 277*:12 (500:9), dpp^{d6} wg^{CX3}/dpp^{d12} wg^{CX4} 0:72* (516:14), dpp^{d6} Dll^{M}/dpp^{d12} Dll^{M} 177*:41 (570:12), dpp^{d6} Dll^{7}/dpp^{d12} Dll^{7} 81*:66 (512:44), dpp^{d6} Dll^{IB}/dpp^{d12} Dll^{IB} 36:95* (445:11), wg^{CX3} Dll^{M}/wg^{CX4} Dll^{M} 0:168* (537:16), wg^{CX3} Dll^{7}/wg^{CX4} Dll^{7} 0:178* (498:27), wg^{CX3} Dll^{IB}/wg^{CX4} Dll^{IB} 0:130* (287:25). The rationale for using heteroallelic genotypes is explained in the Results. Legs were dissected in 70% ethanol, mounted in Faure's solution (Lee and Gerhart 1973) between cover slips, and observed at 400× magnification with an Olympus BH-2 compound microscope. For each genotype 48 male forelegs were analyzed, except for double mutants containing Dll^{7} and Dll^{IB} where N = 20 male forelegs per genotype. To confirm our anatomical findings with dpp^{d6}/dpp^{d12}, we examined 60 previously mounted legs from pharate adults homozygous for the Class-3 mutation dpp^{d2} (6 legs/fly: 3 males, 7 females); pooled data from this sample are reported in the text for leg truncations and tarsal segmentation. For most genotypes, the pupal cuticle was retained during mounting to prevent loss of fragile parts, and extreme care was exercised in handling *wg Dll* legs because the few remaining leg segments are feebly attached.

Mapping of abnormalities. The leg disc fate map in Fig. 1c is based upon the map of Schubiger (1968). The cardinal points D, V, A, and P indicate (future) faces of the *leg* when straightened in a spread-eagle posture relative to the *adult* body (Grimshaw 1905; Hannah-Alava 1958). In contrast, the terms "medial, lateral, upper, and lower", which correspond to left, right, upper, and lower in Fig. 1c, denote parts of the *disc* relative to the *larval* body (Schubiger 1968). The hinges of most leg segments bend only in the DV plane, giving the leg a natural plane of symmetry separating its anterior and posterior faces. Because the edge bristle of the trochanter lies precisely on the dorsal midline of the leg, it was used here to define the DV line in the fate map, with the claws as the other reference point. The DV line, thus defined, approximates, but does not coincide with (1) the boundary separating the A and P lineage compartments of the adult leg, since that boundary is offset posteriorly from the DV plane by about one bristle row (e.g., the edge bristle is in the anterior compartment though the boundary still bisects the claws; Steiner 1976; Held 1979b; Lawrence et al. 1979), nor (2) the edge of the *engrailed*-expressing domain in the leg disc, which is oriented diagonally in the center of the disc but bends vertically toward the stalk (top) as it approaches the dorsal periphery (Brower 1986; Baker 1988; Masucci et al. 1990; Raftery et al. 1991). Our diagonal placement of the DV axis is consistent with Peifer et al. (1991) but not with the maps of other authors (Struhl and Basler 1993: their Figs. 3B, 8; Cohen and Di Nardo 1993) who have depicted the DV axis as a vertical line intersecting the stalk. A second issue regarding the ascribing of axes within the map is where to place the claws relative to the intersection of the DV and AP axes. Bodenstein (1941) and Schubiger (1968) localized the precursor cells for the claws to a site that is dorsal of the disc center (in the upper lateral quadrant). As reported below, we likewise find that the claws indeed behave as dorsal structures since they are absent in V/V tarsal duplications (dpp^{d6}/dpp^{d12}) and duplicated in D/D leg duplications (wg^{CX3}/wg^{CX4}). Schubiger's (1968) map does not include the tarsal bristle rows, which Hannah-Alava (1958) first described and numbered. Following the convention of Girton (1982), we have marked the presumed locations of these rows (as spokes) relative to the the DV line. We have spaced them uniformly because they are arranged at regular intervals on adult tarsal segments (Hollingsworth 1964; Held 1979a) except for the foreleg and hindleg basitarsi where the row 7–8 and 1–2 regions, respectively, are expanded by the transverse rows (Hannah-Alava 1958). [*N.B.*: The nomenclature of bristle rows in Struhl and Basler (1993; their Figs. 3C, 5C, and 5F) is inconsistent with the original chaetotaxy (Hannah-Alava 1958) and erroneous (Struhl, personal communication).] Also added to the map are the tarsal hairs between rows 1 and 8, the pairs of campaniform sensilla that straddle the dorsal midline on tarsal segments T1 and T3 (Russell et al. 1977; Held et al. 1986), and the two macrochaetes (large bristles) on the distal tibia (the apical and preapical bristles). Finally, we have plotted the four ball-and-socket articulations (hinges) of the tarsus as arising from the dorsal midline because partial tarsal joints are characteristically located along this line in the adult leg, even when the remainder of the intersegmental membrane is missing in various mutants (Held et al. 1986). This positioning is confirmed, as reported below, by the symmetrical duplication of these hinges in D/D duplicated legs (wg^{CX3}/wg^{CX4}). Proximal markers (sensilla groups, joints, etc.) of Schubiger's fate map (not diagrammed in Fig. 1c) were also analyzed in our study of mutant phenotypes (data not shown) and were generally duplicated and deficient as indicated in Fig. 3. However, circumferential deletions in the trochanter extended more dorsally in wg^{CX3}/wg^{CX4} and dpp^{d6} wg^{CX3}/dpp^{d12} wg^{CX4} than is indicated in these schematics. For details of wild-type leg anatomy see Figs. 1a, 2a, and Bryant (1978).

Results

In the following survey of leg abnormalities, all statistics refer to the male foreleg, except for dpp^{d2} (see Materials and methods). Second and third legs show defects similar to the forelegs but at different frequencies (as is also true for females *vs.* males). Gross aspects of the single-mutant phenotypes have been described previously (Spencer et al. 1982; Sato 1984; Sunkel and Whittle 1987; Baker 1988; Bryant 1988; Cohen and Jürgens 1989).

Single mutant phenotypes

decapentaplegic. Mutations at the *dpp* locus are categorized based upon their phenotypes and lethal phases (Spencer et al. 1982; Gelbart 1989). Class-3 alleles permit metamorphosis and cause truncations of various appendages – and dpp^{d6} is typical. Because the genetic lesions involve rearrangements (St. Johnston et al. 1990; Lindsley and Zimm 1992) whose other breakpoint (if associated with a mutation) could cause complications when homozygous, dpp^{d6} was studied in heteroallelic combination with dpp^{d12}, a Class-5 allele (Class-3/Class-5 genotypes yield Class-3 phenotypes). All legs from dpp^{d6}/dpp^{d12} adults lack claws and dorsal tarsal structures, including the sensilla campaniformia on segments T1 and T3 and the ball-and-socket hinges between adjacent segments (Fig. 2d; cf. Held et al. 1986). In tarsal segments T4 and T5, the missing dorsal structures are commonly replaced with mirror-image copies of ventral structures – a "V/V" phenotype. In 35% of the forelegs the basitarsus has such a V/V duplication in its sex comb (Fig. 2c). The average number of "teeth" (thickened bristles) in these V/V combs is 17.2 (SD = 1.6, $N = 17$; in a random sample regardless of comb type, $\bar{x} = 16.3$, SD = 2.4, $N = 20$), compared with 11.4 (SD = 1.0, $N = 20$) for the wild type. Segments proximal to the tarsus are relatively normal, though V/V duplications can extend into the tibia (common in dpp^{d2}). On the second leg, where bristle rows are more clearly identifiable, the plane of symmetry in such mirror-symmetric "Janus" basitarsi typically runs along rows 2 and 7 (Fig. 3). When there is no pattern duplication, the dorsally deficient ("V/–") tarsus is shortened and often curled dorsally (Fig. 2d) – giving the illusion of a truncation when in fact all segments are present. Thus, neither the V/V nor the V/– phenotype of dpp^{d6}/dpp^{d12} is technically "distally deficient" (Spencer et al. 1982) except for missing claws, and even this trait may not originate as a distal defect (see below). (For the stronger allele dpp^{d2}, which manifests the same V/V and V/– syndrome, truncations were found in 31 out of 60 legs, ending in segments ranging from the tibia to T4.) Partial fusions of segments are frequent at the joints T2/T3 and T4/T5 (85%, 73%), less so at T1/T2 and T3/T4 (52%, 42%). (In dpp^{d2}, T2/T3 and T4/T5 fusions are 5 times more common than T1/T2 and T3/T4 fusions: 23 and 26 cases *vs.* 5 and 5.) Segment T3 (and less so T2) often tapers distally or is thin along its entire length.

wingless. The mutation wg^{CX3} is unusual among *wg* alleles insofar as it affects the legs in addition to the wings (Lindsley and Zimm 1992). Because it is a small deletion (3′ to the transcript domain) that may remove other genes (Baker 1987, 1988), it was studied in heteroallelic combination with the null allele wg^{CX4} (a deletion at the 5′ end of the gene). The wg^{CX3}/wg^{CX4} genotype causes a loss of ventral structures and a mirror-image duplication of dorsal ones – a "D/D" phenotype. In contrast to dpp^{d6}/dpp^{d12}, the duplication usually (69% of the forelegs studied) affects the entire leg instead of only the tarsus (Fig. 2b; cf. Peifer et al. 1991). There are typically two pairs of mirror-image claws (rarely fewer), two sets of mirror-image tarsal hinges, and duplicate pairs of sensilla on T1 and T3. Furthermore, the tibia is constricted near its proximal end, and the sex comb is reduced to an average of only 3.8 teeth (SD = 2.5, $N = 20$) which point distally rather than ventrally. Evidently, the normal 90° rotation of the sex comb (Tokunaga 1962) fails to occur. Curiously, the D/D Janus phenotype of wg^{CX3}/wg^{CX4} has the same plane of symmetry as the V/V dpp^{d6}/dpp^{d12} pattern: it also tends to coincide with rows 2 and 7 (Fig. 3). Because the deficient sector reported for wg^{CX3}/wg^{CX4} by Baker (1988; his Fig. 5) seems to differ slightly (bounded by rows 1 and 7?), we also studied second-leg basitarsi which offer greater resolution because the rows are easier to recognize (Held 1979a). Among eight cases of Janus basitarsi whose complete chaetotaxy was analyzed, two had a single complete row 2 and 7 exactly at the symmetry plane, three had a single row 2 but a partially duplicated row 7, and three had a partially duplicated row 2 and row 7. Thus, for this segment the symmetry plane does indeed intersect rows 2 and 7, and the deficient sector is centered on the ventral midline, where *wg* is apparently expressed (Fig. 1d; cf. Peifer et al. 1991). Aside from the purely D/D legs, another 6% of the legs are D/D but are truncated in the tarsus; 10% are D/D from the coxa to usually the femur or tibia where a normal pattern appears (continuing to the tip of the leg) with a small single-segment sidebranch at the transition point; and the remaining 15% are D/D from the coxa to the tibia or a tarsal segment where they branch to become 2 complete (or 1 normal and 1 D/D) distal patterns. Unlike dpp^{d6}/dpp^{d12}, wg^{CX3}/wg^{CX4} tarsi do not exhibit segment fusions.

Distal-less. Three different *Dll* mutations were used, none of which is a null allele (Cohen and Jürgens 1989). The mildest allele, Dll^{M}, causes (1) elimination (in 60% of the legs) of the edge bristle on the trochanter and (2) partial fusions of tarsal segments at the T3/T4 (25%) or T4/T5 (23%) joints or rarely (4%) at the tibia/T1 joint. Tibia/T1 fusion is greater in females, especially in the hindlegs (cf. Sato 1984). In 4 of 20 female second legs examined, extra inverted joints were found in T3 or T4 (cf. Held et al. 1986). The alleles Dll^{7} and Dll^{IB} have stronger effects: the edge bristle and the claws virtually disappear, partial segment fusions occur at an 80%-or-greater frequency at the trochanter/femur, tibia/T1, T2/T3, T3/T4, and T4/T5 joints (T1/T2 fusions: 20% and 35% for Dll^{7} and Dll^{IB} respectively), and the tarsus is shortened (T4 is nearly eliminated) though remnants of all segments remain. Along the DV axis the only asymmetric effects are: (1) removal of the (mid-dorsal) edge bristle and (2) a tendency for tibia/T1 fusions to occur on the dorsal side of the leg.

Double mutant phenotypes

wg Dll. Compounds of *wg* with *Dll* have drastically truncated, mirror-image D/D legs. For wg^{CX3} Dll^{M}/wg^{CX4} Dll^{M} all proximal segments are reduced in size, and the leg typically ends with a partial tibia. (Second and third

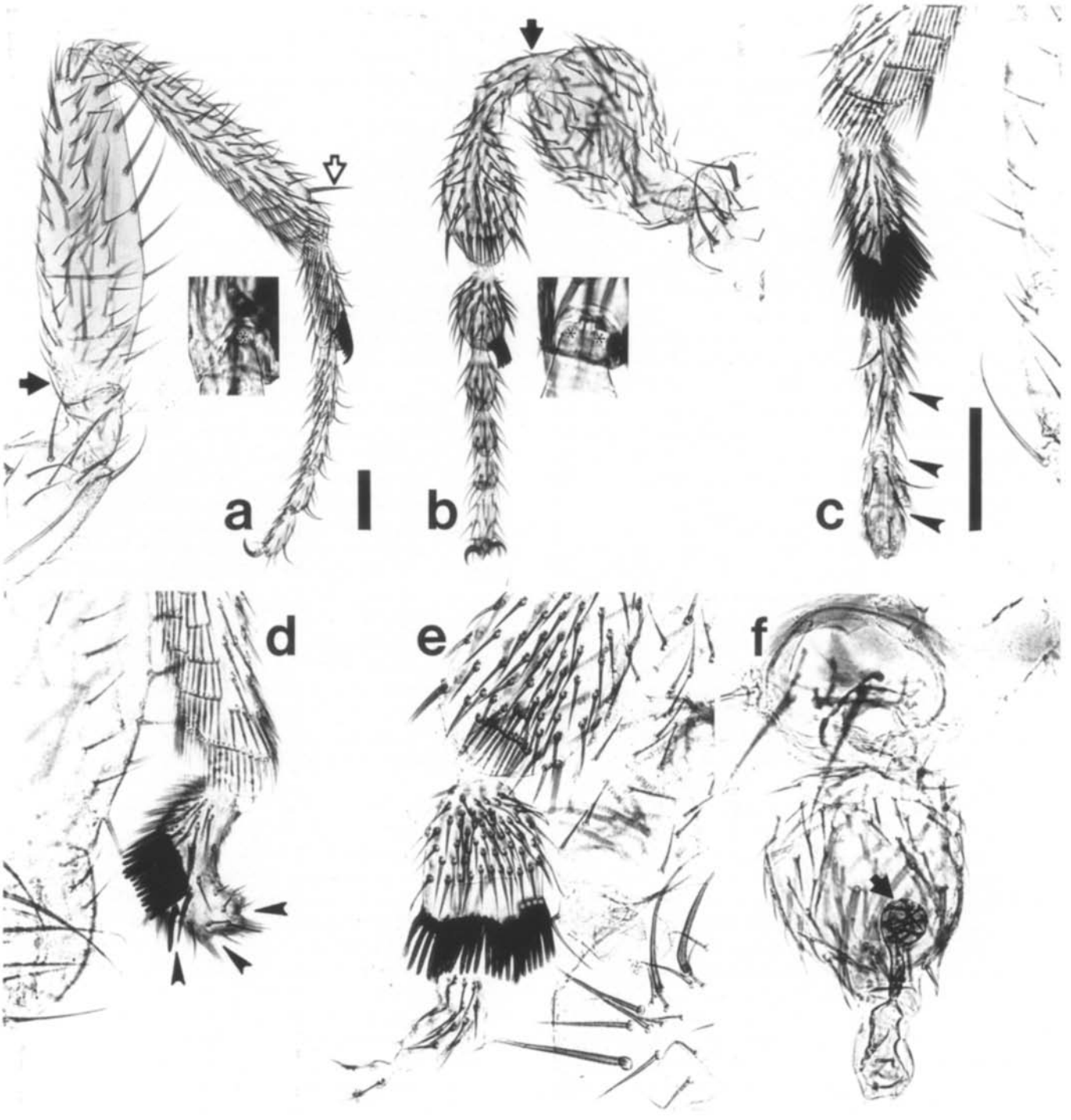

Fig. 2a–f. Abnormal leg phenotypes caused by *dpp*, *wg*, and *Dll* mutations and their compounds. **a** Left foreleg from a wild-type male, viewed from the anterior. This picture corresponds to the right half of Fig. 1a, with the leg bent at the femur-tibia joint so that the edge bristle (*solid arrow*) is pointing up instead of down (*unfilled arrow marks the preapical bristle*). The outer edge is the dorsal midline, except that the basitarsus has turned (due to sandwiching between cover slips) so that its sex comb – an anterior structure (the vertical row of dark bristles) – is along the outer edge. Unlike Fig. 1 where the claws are conventially drawn pointing outwards, here they point in their natural ventral direction. Bar length in **a** (same magnification as **b**) and **c** (same magnification as **d–f**) is 100 μm. The inset (additional 4× magnification) shows the ball-and-socket articulation (*asterisk marks the condyle*) between T1 and T2. **b**, **c** Mirror-image *Janus* phenotypes (cf. Frankel 1989) for wg^{CX3}/wg^{CX4} (**b**) and dpp^{d6}/dpp^{d12} (**c**). The right foreleg in (**b**) manifests D/D mirror-image symmetry: the tibia bears a preapical bristle on each side (only one of the duplicate edge bristles is in focus). Associated defects include 2 pairs of claws, fewer sex comb teeth (the comb has failed to rotate vertically), bulbous segments, and a constriction (*arrow*) near the base of the tibia. The inset (extra 4× magnif.) shows the double hinges (*asterisks mark the balls*) between T1 and T2. In (**c**) a right dpp^{d6}/dpp^{d12} tarsus is shown at higher magnification. Note its V-shaped sex comb (a V/V duplication) and the absence of claws. *Arrows* point to *joints* between tarsal segments. Ball-and-socket articulations are absent, and the only intersegmental membranes that encircle the entire circumference are at T1/T2 (obscured by the sex comb) and

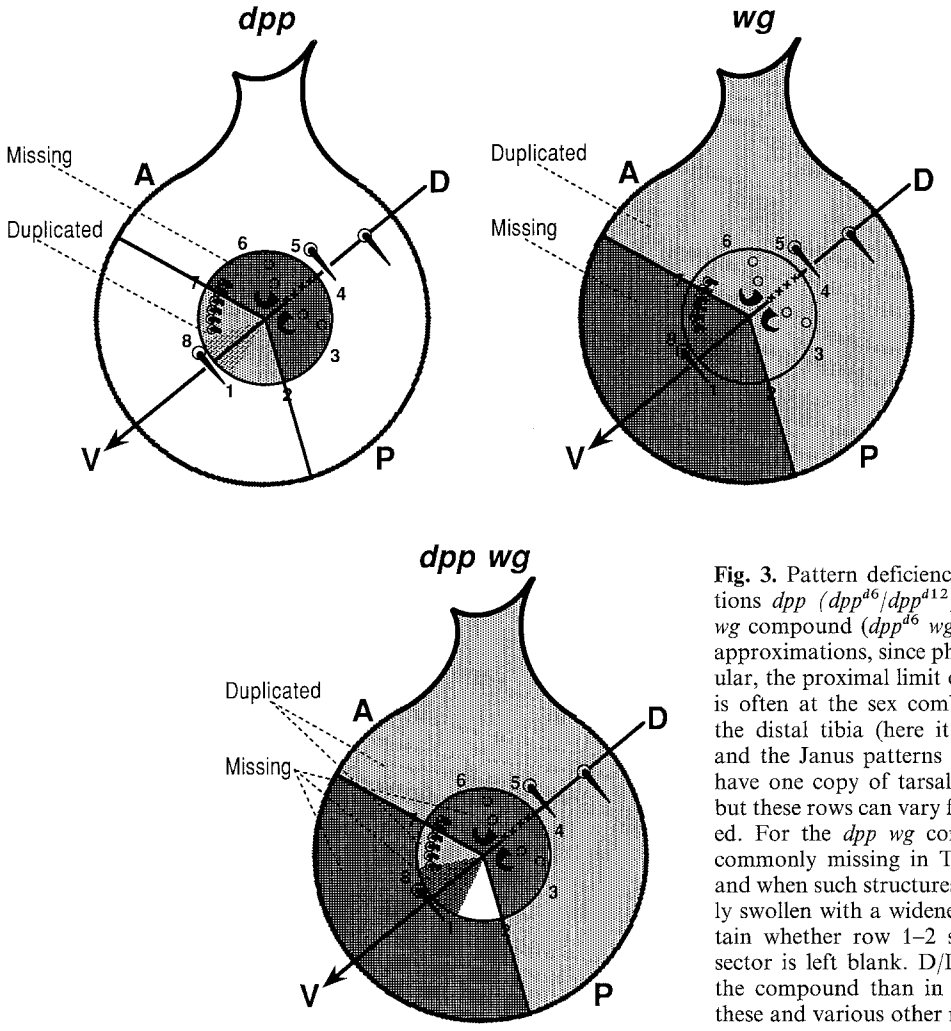

Fig. 3. Pattern deficiencies and duplications caused by the mutations *dpp* (dpp^{d6}/dpp^{d12}) and *wg* (wg^{CX3}/wg^{CX4}) and by the *dpp wg* compound ($dpp^{d6}\ wg^{CX3}/dpp^{d12}\ wg^{CX4}$). Shaded areas are only approximations, since phenotypes of individual legs vary. In particular, the proximal limit of V/V (*double-ventral*) duplications in *dpp* is often at the sex comb (distal basitarsus) but may extend into the distal tibia (here it is drawn as the tibia/tarsus boundary), and the Janus patterns of both *dpp* (V/V) and *wg* (D/D) usually have one copy of tarsal rows 2 and 7 at the plane of symmetry, but these rows can vary from partially missing to partially duplicated. For the *dpp wg* compound, midventral structures are more commonly missing in T2 and T3 than in other tarsal segments, and when such structures are missing from T1 the segment is usually swollen with a widened sex comb (Fig. 2e). Because it is uncertain whether row 1–2 structures are duplicated in *dpp wg*, this sector is left blank. D/D proximal segments are less common in the compound than in wg^{CX3}/wg^{CX4}. See text for frequencies of these and various other minority phenotypes

T3/T4 (*middle arrow*); T2/T3 and T4/T5 manifest vestigial indentations on the ventral (*right*) edge. Segment T3 tapers distally. **d** Distal tibia and tarsus of a left dpp^{d6}/dpp^{d12} leg, which exhibits a "V/−" phenotype. Dorsal structures (inclu. hinges and sensilla) are missing, but ventral structures are not duplicated. Despite the reduced size of the tarsus, it is not "distally deficient" (cf. Spencer et al. 1982) since the normal number of (albeit partial) joints can still be discerned on the ventral face (*arrows*; T1/T2 is obscured by the sex comb). **e** Part of a right leg from the *dpp wg* double mutant $dpp^{d6}\ wg^{CX3}/dpp^{d12}\ wg^{CX4}$. Note the unrotated, widened sex comb, which wraps around to the other side of the segment (the 4 bristles on the other side are marked by asterisks). Such severely affected basitarsi are not only missing dorsal structures but ventral ones as well including: (*1*) the stout bristles typical of row 1, (*2*) the *central bristle* (Hannah-Alava 1958) corresponding to row 8, and (*3*) the transverse rows, whose bristle sockets characteristically osculate (cf. Fig. 2a). In this case, the basitarsal circumference is swollen to about twice its normal size, though the diameters of distal segments are normal. **f** Leg remnant from a $wg^{CX3}\ Dll^{M}/wg^{CX4}\ Dll^{M}$ pharate adult. This D/D symmetric leg is truncated in the proximal tibia, at about the same level as the constriction typical of wg^{CX3}/wg^{CX4} legs (**b**), but here the tibial vestige is turned inside-out as an ingrowth (note the inward-pointing bristles) extending from the constriction site to a point about a third of the way into the femur (*arrow*)

legs are less affected.) Strangely, in 12 of the 48 forelegs examined for this genotype, the tibial remnant resides *inside* the distal end of the femur (Fig. 2f). Such ingrowths contain as many as 28 bristles on the inner surface (corresponding to the outer surface for a wild-type tibia). In more proximal segments, there were 18 (total) cuticular vesicles, containing bristles, sensilla trichodea, or hairs. Attachments between the femur and trochanter are fragile or nonexistent. Bristles on several detached femurs have a light pigmentation suggestive of an earlier severing of segmental connections (which would have cut off the blood supply and hence prevented full differentiation; cf. Bryant et al. 1988). For $wg^{CX3}\ Dll^{7}/wg^{CX4}\ Dll^{7}$ and $wg^{CX3}\ Dll^{1B}/wg^{CX4}\ Dll^{1B}$ the vestigial leg also has a D/D anatomy and is usually truncated at the coxa/trochanter or trochanter/femur joint.

dpp Dll. The legs of $dpp^{d6}\ Dll^{M}/dpp^{d12}\ Dll^{M}$ flies exhibit all of the traits seen in dpp^{d6}/dpp^{d12}, plus a 100% absence of the edge bristle – an exaggeration of the 60% loss seen in Dll^{M}. In a few (8/48) cases the leg terminates at the tibia/T1 joint or more distally. Such truncations occur in all legs from *dpp* compounds with Dll^{7} or Dll^{1B}. In $dpp^{d6}\ Dll^{7}/dpp^{d12}\ Dll^{7}$ the femur is shortened and the tibia reduced to a stub. In $dpp^{d6}\ Dll^{1B}/dpp^{d12}\ Dll^{1B}$

the femur is also short, but a larger tibial remnant now displays a mirror-image V/V pattern, and the leg either ends there (40%) or in the proximal tarsus (60%). Both of the latter compounds lack edge bristles and exhibit trochanter/femur fusions ($\geq 95\%$).

dpp wg. In dpp^{d6} wg^{CX3}/dpp^{d12} wg^{CX4} double mutants the legs show a curious mixture of *dpp* and *wg* characters, with some novel features as well. The tarsi are *dpp*-like insofar as they lack claws, ball-and-socket articulations, and dorsal sensilla, but *wg*-like in their rarity of tarsal segment fusions (only 2 cases in 48 legs). The sex combs are enlarged even more than in dpp^{d6}/dpp^{d12}, with an average of 26.9 teeth per comb (SD = 4.1, $N = 20$) but fail to rotate on the dorsal surface (rotation on the ventral side is variable) as in wg^{CX3}/wg^{CX4}. In some cases the sex comb stretches around nearly 70% of the circumference (Fig. 2e). Except for the sex comb, there is no evidence of the V/V duplications seen in dpp^{d6}/dpp^{d12}. Segments T2 and T3 are thinner than in dpp^{d6}/dpp^{d12} and lack the diagnostic bristles and hairs of the ventralmost region. The proximal segments are either *wg*-like (a D/D coxa with continuation of the D/D pattern to various levels, usually through the tibia; 58%) or wild-type (42%).

Discussion

Significance of the Janus phenotypes

The double-dorsal leg phenotype caused by wg^{CX3} was described by Baker (1988) and Peifer et al. (1991), and the double-ventral duplication caused by Class-3 *dpp* mutations was reported by Spencer et al. (1982) and Bryant (1988), though in the latter case the location of the plane of symmetry was not identified. Surprisingly, we find that the symmetry plane is virtually the same in both the D/D and V/V phenotypes (Fig. 3). Intersecting rows 2 and 7, it partitions the fate map into a wedge (central angle $\approx 135°$) centered on the ventral midline and its complement (225°) centered on the dorsal midline. Neither area is a lineage compartment (Steiner 1976). The only special property ascribed to them is that they each form the base for a different type (converging *vs.* diverging) of triplicated (branched) leg in a heat-sensitive cell-lethal mutant (Girton 1981).

Struhl and Basler (1993) forced the wg^+ gene to be expressed ectopically in the dorsal half of the leg disc where it induces ventral elements in surrounding (genetically wild-type) tissue. This result proves that wg^+ can emit a signal that specifies ventral cell fates. The wg^+ product is a member of the *Wnt* family of growth factors (Nusse and Varmus 1992) and is secreted in the *Drosophila* embryo (van den Heuvel et al. 1989; González et al. 1991). The 135° sector of the fate map that is missing in wg^{CX3}/wg^{CX4} can thus be interpreted as the group of cells which need secreted wg^+ product in order to adopt a ventral fate (Struhl and Basler 1993). In its absence they would adopt a dorsal fate, thereby giving the leg a D/D Janus anatomy.

Could the dpp^+ gene be functioning in a similar capacity for the dorsal 225° sector? Like *wg*, *dpp* encodes a diffusible member of a growth factor family – in this case the TGF-β family (Padgett et al. 1987; Panganiban et al. 1990) – but unlike *wg* it is expressed a stripe along the entire DV axis (Fig. 1d). To endow *dpp* with a *wg*-like role, it would be necessary to assume that expression in the ventral half-stripe is nonfunctional, and indeed transcription there is less than in the dorsal half-stripe (Masucci et al. 1990). Inhibition of ventral *dpp* function could be mediated by *wg* – a conjecture made plausible by (1) the interaction of these genes at the inception of the disc (Cohen et al. 1993) and (2) interactions between these growth factor families along the DV axis in *Xenopus* embryos (Sokol and Melton 1992; Christian and Moon 1993). In *Drosophila* limb development *dpp* has been thought to act along the proximodistal axis since appendages are often truncated in *dpp* mutants (e.g., in dpp^{d2} legs but not for the more typical Class-3 mutation dpp^{d6}; Gelbart 1989; Wilkins and Gubb 1991; Williams and Carroll 1993). However, this idea is "an oversimplification" (Spencer 1982). The "V/−" phenotype (dorsal structures missing but no ventral duplication) contradicts it, and the absence of claws in Class-3 legs could just as easily signify a dorsal deficiency given their eccentric location in the fate map (Bodenstein 1941; Schubiger 1968). We propose that the dpp^+ product specifies cell positions relative to the dorsal midline in the leg disc. This hypothesis envisions a role for *dpp* analogous to its role in the early embryo where it specifies fates within the dorsal 40% of the ectoderm relative to the dorsal midline (Ray et al. 1991; Ferguson and Anderson 1992a, b; Wharton et al. 1993).

If *dpp* and *wg* do play comparable roles, then why don't they manifest similar syndromes? Chief among the differences is the "V/−" phenotype in dpp^{d6}/dpp^{d12} (Fig. 2d), which has no "D/−" counterpart in wg^{CX3}/wg^{CX4}. Reductions in function of these genes (Baker 1988; St. Johnson et al. 1990) thus seem to have different effects: a transformation of cell states (*wg*) *vs.* a removal of tissue (*dpp*) which may (V/V) or may not (V/−) provoke a duplication. Conceivably, the dpp^+ product plays a trophic as well as a morphogen role i.e., it provides an essential growth-promoting signal (cf. Cross and Dexter 1991). Consistent with this idea, (1) cells along the dorsal midline must be viable in order for the entire disc to survive (Postlethwait and Schneiderman 1973; Russell et al. 1977), and (2) *dpp* is one of the first genes activated during regeneration (Brook et al. 1993). Extensive cell death has been found in *dpp* mutant discs (Bryant 1988; Masucci et al. 1990), but cell death seems not to be a factor in *wg* duplications (Morata and Lawrence 1977; James and Bryant 1981; Williams et al. 1993). Necrosis of dorsal cells in *dpp* leg discs could cause V/V duplications by removal of a large portion (225°) of the circumference and stimulation of intercalation via the shortest route, as dictated by the Polar Coordinate Model of French et al. (1976). However, the notion of morphogen (*wg* and *dpp*) sources does not easily fit their model, which invokes local interactions (cf. Held 1992).

A "deficiency-without-duplication" phenotype is also found in the eyes of $dpp^{d\text{-}blk}$ mutants (Masucci et al. 1990), but in that case the *ventral* half of the organ is missing – an apparent anomaly until it is remembered that the eye undergoes a 180° rotation during development which reverses its DV axis (Struhl 1981). Contrary to expectation, *dpp* mosaic wings exhibit a wild-type phenotype only if dpp^{null} clones reside outside the ventral and dorsal areas just anterior to the A/P compartment boundary (Posakony et al. 1991): if our hypothesis for the leg also applied to the wing, then *dpp* malfunction should only be a problem for the dorsal half-stripe.

A second key difference between the *wg* and *dpp* leg syndromes is that wg^{CX3}/wg^{CX4} affects the entire leg, whereas dpp^{d6}/dpp^{d12} primarily affects the tarsus. The explanation may be that different parts of the *dpp* stripe are controlled by different enhancers, and Class-3 mutations affect only a subset (Masucci et al. 1990; St. Johnston et al. 1990; Blackman et al. 1991). The regional specificity of the enhancers may also explain why some joints (T2/T3, T4/T5) tend to be more defective than others (T1/T2, T3/T4). If the dpp^{+} product does function as a trophic factor, then its entire removal should stifle disc growth as seen in Class-5 mutants (Spencer et al. 1982). The ability of Class-5 discs to be rescued in mixed implants with wild-type tissue (Bryant 1988) supports the notion that the rescuing factor is diffusible over distances of many cell diameters.

Double mutant phenotypes

wg Dll. *Dll* mutations interact synergistically with wg^{CX3}/wg^{CX4}. The most dramatic illustration is the *wg* Dll^{M} compound which has severely truncated legs (Fig. 2f), despite the fact that Dll^{M} alone has a nearly wild-type foreleg phenotype. Because *Dll* function depends upon *wg* activity at the inception of the leg disc (Cohen et al. 1993), the combination of reduced *wg* function with even slightly reduced *Dll* function might be sufficient to disrupt establishment of the proximodistal axis, and the mirror-image D/D condition of the disc could prevent later recovery through distal regeneration (cf. Bryant et al. 1981). The ingrown tibiae of wg^{CX3} Dll^{M}/wg^{CX4} Dll^{M} flies are attributable to a tibial constriction also found in wg^{CX3}/wg^{CX4} single mutants (Fig. 2b): a "pursestring" contraction of this annular region of the leg disc could prevent the folded epithelium from everting past the blockage, hence forcing it to elongate backwards into the femur. Similar ingrowths have been described for *fat* mutants (Bryant et al. 1988) which also exhibit cuticular vesicles like those of wg^{CX3} Dll^{M}/wg^{CX4} Dll^{M}, suggesting a common flaw in epithelial integrity. The wg^{CX3} Dll^{M}/wg^{CX4} Dll^{M} compound has another constriction at the trochanter-femur joint, but the outcome there appears to be eversion (with normal polarity) and subsequent detachment, instead of a reversed polarity ingrowth. Truncation at the coxa, frequent in wg^{CX3}/wg^{CX4} compounds with Dll^{7} and Dll^{1B} is the null phenotype for the *Dll* locus (Cohen et al. 1993), implying that all *Dll* function has been eliminated in these genotypes.

dpp Dll. A milder interaction was observed for combinations between *dpp* and the *Dll* alleles Dll^{7} and Dll^{1B}: truncations occur at more proximal levels than with the *Dll* mutations alone. This synergy may be due to a shared function in the dorsal half of the disc. The dorsal bias of *dpp* function has been discussed above; for *Dll* a weak dorsal bias is evident in its removal of the mid-dorsal trochanter edge bristle. The Dll^{+} gene encodes a homeodomain protein (Cohen et al. 1989), implying a function as a nuclear transcription factor. Hence, the interaction might be due to the *Dll* protein binding to *dpp* enhancer elements that control specific leg segments, thereby coupling radial (*Dll*) and angular (*dpp*?) variables of the presumptive coordinate system.

dpp wg. The tarsi of dpp^{d6} wg^{CX3}/dpp^{d12} wg^{CX4} are more *dpp*-like than *wg*-like, indicating a partial epistasis there of *dpp* over *wg*, though the D/D duplications characteristic of *wg* are still asserted proximally. The swelling of the basitarsus is attributable to the greater number of sex comb teeth plus the failure of the sex comb to rotate. The greater number of teeth (27 on average *vs.* 16 in dpp^{d6}/dpp^{d12}), in turn, may be due to a biasing of the remaining coordinates away from the ventral midline, since the ventralmost bristle rows are missing (also the case for tarsal segments T2 and T3). Finally, this biasing may be due to the fact that intermediate levels of *wg* activity lead to ventrolateral (*vs.* midventral) pattern elements (Struhl and Basler 1993). Why wouldn't a reduced level of wg^{+} trigger a D/D duplication in the tarsus as in the single mutant? Perhaps, as conjectured above, wg^{+} suppresses dpp^{+} function in the ventral region, and D/D duplications are actually caused by derepression of dpp^{+} in its ventral half-stripe. In that case, the absence of a D/D duplication in the tarsus could be due to an inability of mutated *dpp* enhancers to activate dpp^{+} expression ventrally when repression (by wg^{+}) is removed.

The imaginal disc coordinate system

The Polar Coodinate Model of French et al. (1976) has recently been buttressed by the finding that many genes are expressed in sectors or annuli within the leg disc (Bryant 1993). Genes that specify (by intercellular communication) or encode (by intracellular "memory") the coordinate variables should mutate to cause transformations along their respective axes. Wilkins and Gubb (1991) proposed that the "segment polarity" class of embryonic segmentation genes might specify positions around the circumference of each disc, though a recent study (Held 1993) failed to uncover any inter-sector transformations using adult-viable alleles of these genes. Inter-sector transformations reported previously for *wg* and *dpp* mutants have been analyzed here, and the results, together with the known domains of gene expression, seem to indicate two morphogen sources at opposing ends of the dorsoventral axis. Whether such a mechanism can be accommodated within the framework of a polar coordinate system must await further work, as

must an understanding of how coordinates along the proximodistal axis (possibly encoded by *Dll*) depend upon the (DV?, angular?) coordinates specified by *dpp* and *wg*.

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