Axes, boundaries and coordinates: the ABCs of fly leg development

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Summary

Recent studies of gene expression in the developing fruitfly leg support a model – Meinhardt's Boundary Model – which seems to contradict the prevailing paradigm for pattern formation in the imaginal discs of *Drosophila* – the Polar Coordinate Model. Reasoning from geometric first principles, this article examines the strengths and weaknesses of these hypotheses, plus some baffling phenomena that neither model can comfortably explain. The deeper question at issue is: how does the fly's genome encode the three-dimensional anatomy of the adult? Does it demarcate territories and boundaries (as in a geopolitical map) and then use those boundaries and their points of intersection as a scaffolding on which to erect the anatomy (the Boundary Model)? Or does it assign cellular fates within a relatively seamless coordinate system (the Polar Coordinate Model)? The existence of hybrid Cartesian-polar models shows that the alternatives may not be so clear-cut: a single organ might utilize different systems that are spatially superimposed or temporally sequential.

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How did arthropods acquire segmented legs?

Arthropods are so-named because of their jointed legs. The evolution of arthropods from legless annelid-like ancestors involved the acquisition of segmented, cylindrical appendages that superficially resemble the segmented, cylindrical body[1]. Could leg segmentation have arisen by the heterotopic deployment (in nascent leg buds) of a preexisting annelid program for body segmentation? The 40 genes (approximately) that mediate *Drosophila* body segmentation fall into three categories based upon their zygotic expression patterns and mutant phenotypes: 'gap', 'pair-rule' and 'segment-polarity'[2]. If the processes of body and leg segmentation are truly homologous, then they should use the same genes. However, only two body segmentation genes have so far shown any evidence of also helping to establish leg segment boundaries: *odd-skipped*, a pair-rule gene, is expressed in a subset of leg segments at their distal ends[3] and *dishevelled*, a segment-polarity gene, mutates to produce a 'segment-polarity' phenotype of duplicated inverted joints in the tarsi of all six legs[4]. It therefore seems unlikely that the arthropod leg originated as a miniature version of the segmented body column. Nevertheless, one group of body segmentation genes – the segment-polarity class – does appear to be instrumental in constructing other features of leg anatomy that are unrelated to metamerism *per se*.

Ascendancy of the 'seamless' polar coordinate paradigm

The core problem of developmental biology is how embryonic cells adopt particular fates that are appropriate for their positions. An insightful idea for solving this problem was formulated by Wolpert in 1969 as the 'Positional Information' Hypothesis[5]. Development, he argued, proceeds in two stages. First, cells within a definite area ('field') collectively establish some kind of coordinate system within which every cell could 'know' its position. For example, a chemical signal ('morphogen') might be secreted by cells along one edge of the array, and the dilution of that chemical as it diffused away from the source would lead to a concentration gradient, enabling a cell to gauge its distance from the edge by measuring the concentration of morphogen at its location (just as you can estimate your distance from a train by the faintness of the horn). In the second stage of the process, each cell would 'interpret' its coordinates as a specific fate (just as a postman can deduce your city based upon your postal code). In principle, embryos could use any type of coordinate system, though Cartesian systems seemed sim-

plest. For a three-dimensional organ, there would presumably be three perpendicular (x, y and z) gradients. A different morphogen could be used for each axis, or a single morphogen could be used repeatedly, with each cell assessing its three coordinates sequentially.

The fruitfly *Drosophila* has provided a fertile testing ground for Wolpert's hypothesis since its adult appendages develop as separate 'imaginal discs', so-called because they are flat and round and form adult (a.k.a. 'imaginal') structures[3]. The discs grow as hollow epithelial sacs inside the body of the larva until metamorphosis when they come to the surface and fuse into a patchwork quilt that forms the adult integument. Each leg disc, for example, begins as a cluster of about 20 cells on the flank of the embryo, invaginates as a pocket, grows during the larval period until it contains 10^4 cells, turns inside-out during the pupal period, and forms a cylindrical leg by telescoping its concentric folds[3,6]. By transplanting pieces of imaginal discs into blood-filled cavities of host larvae, it was possible to establish 'fate maps' that chart the correspondence between various areas within a disc and the particular parts of the adult that they make (e.g. see Fig. 2b). When fragments of the wing disc were given additional time to grow, it was found that large pieces tend to regenerate missing parts, whereas small pieces duplicate themselves, regardless of their location in the fate map. This result was paradoxical from the vantage point of Cartesian models because it seemed to refute the notion that a fragment must contain a special boundary or reference point (like the peak of a gradient) in order to regenerate.

In 1976 French *et al.*[7] resolved this 'Seamless Regeneration Paradox' by invoking a polar coordinate system in which all cells could signal and receive positional information, with no need for special reference axes. During tissue growth in normal development or in response to surgery, newborn cells would adopt coordinates based upon the (cell surface?) coordinates of preexisting neighboring cells, with no long-distance, morphogen-mediated communication. The presumed coordinates are distance from the disc center ('radial' coordinate) and circumferential location ('angular' coordinate). The angular coordinate is usually drawn as a clockface of numbers from 0 to 12 around the disc (Fig. 5c). Cells are assumed to perceive '0' and '12' as identical values so that there is no seam in the coordinate system along this radius. According to the model, when a fragment is isolated, (1) the wound edges heal together, confronting cells that normally would not be adjacent; (2) the cells along this 'scar' resolve their differences by intercalary growth of new cells that bridge the gap in positional values; and (3) the intercalation follows the shorter of the two possible routes around the circumference. (How cells might 'compute' the shorter route remains a mystery[8].) Thus, the Seamless Regeneration Paradox was now explicable because large fragments should contain more than half of the circumferential coordinates, regardless of their location relative to any imaginable boundary (assuming a uniform density of angular coordinates around the disc).

... but compartment boundaries imply that seams do matter

Ever since it was proposed, the Polar Coordinate Model has reigned as the paradigm for pattern formation in imaginal discs, but it has always been pestered by a nagging riddle: if seams are so irrelevant for disc development, then why do discs possess inviolable internal boundaries? In 1973 Garcia-Bellido *et al.*[9] demonstrated within the wing disc the existence of territories ('compartments') whose boundaries could not be crossed by cells from either side of the boundary, even if marked cells and their clonal descendants were genetically endowed with a growth advantage relative to the background cells. Why should such boundaries exist? Crick and Lawrence[10] conjectured that the boundaries might function as reference axes in a Cartesian coordinate system, with the cells along a boundary producing a morphogen that could specify positional information for the remaining cells of the disc. Whereas the results of pre-1976 regeneration studies had argued against such a system, a subsequent investigation by Karlsson[11] indicated a clustering of the wing disc's angular coordinates along the A/P (anterior/posterior) compartment boundary. Complicating the picture still further, however, is the regenerative behavior of leg disc fragments. A specific quarter of the leg disc (the upper medial; Fig. 3b) can regenerate the remaining three quarters[12], implying a clustering of more than half of the angular coordinates inside this quadrant[7], but, unlike the situation in the wing disc, this piece resides entirely within the anterior compartment. Thus, the A/P compartment boundary seems irrelevant for leg disc regeneration despite its apparent importance in wing disc regeneration[13]. Many attempts have been made to reconcile the disparate facts pertaining to appendage development and compartment boundaries[13]. The most successful has been Meinhardt's 'Boundary Model'[14]. To comprehend why this model has proved to be so useful, the reader must first understand some basic facts about appendage development. The following discussion focuses on the fly leg, whose unique anatomical features serve to highlight the contrasting tenets of the competing models.

A primer on leg anatomy

In *D. melanogaster*, each leg has nine segments that are connected by flexible joints. Like the human elbow, most of the joints bend in only one plane, which defines the dorsal-ventral axis (Figs 1, 2). [Note: in this article, axes and symmetry planes are denoted by a double-headed arrow (e.g. D↔V) and boundaries by a slash mark (e.g. A/P, where A and P are the anterior and posterior halves of the leg); thus,

the D↔V line = the A/P boundary (Fig. 2b).] The bristle pattern of the second-leg tarsus shows a remarkable symmetry about the D↔V plane (Fig. 3a). This symmetry may reflect the evolutionarily ancestral condition for all six legs since the 2nd leg lacks the 1st- and 3rd-leg bristle embellishments (sex combs and transverse rows) that are used in mating and preening – leg functions which presumably evolved after walking[15,16]. The other two natural axes of the leg are the anterior-posterior (A↔P) and proximal-distal (P↔D) axes.

Although three axes imply three dimensions, the leg imaginal disc is more aptly described as a (folded) two-dimensional sheet, since its epithelium is only one cell thick (Fig. 2c)[17]. The A and P halves of the leg are roughly congruent with the A and P compartments, which were originally revealed by clonal analysis[18] and subsequently found to coincide with the expression domains of the segment-polarity genes *cubitus-interruptus* (A compartment) and *engrailed* and *hedgehog* (P compartment) respectively (Fig. 4a)[19,20]. Given the notion that compartment boundaries act as reference axes for specifying positional information[10], it was especially surprising when the A/P compartment boundary was found to be offset by several cell diameters

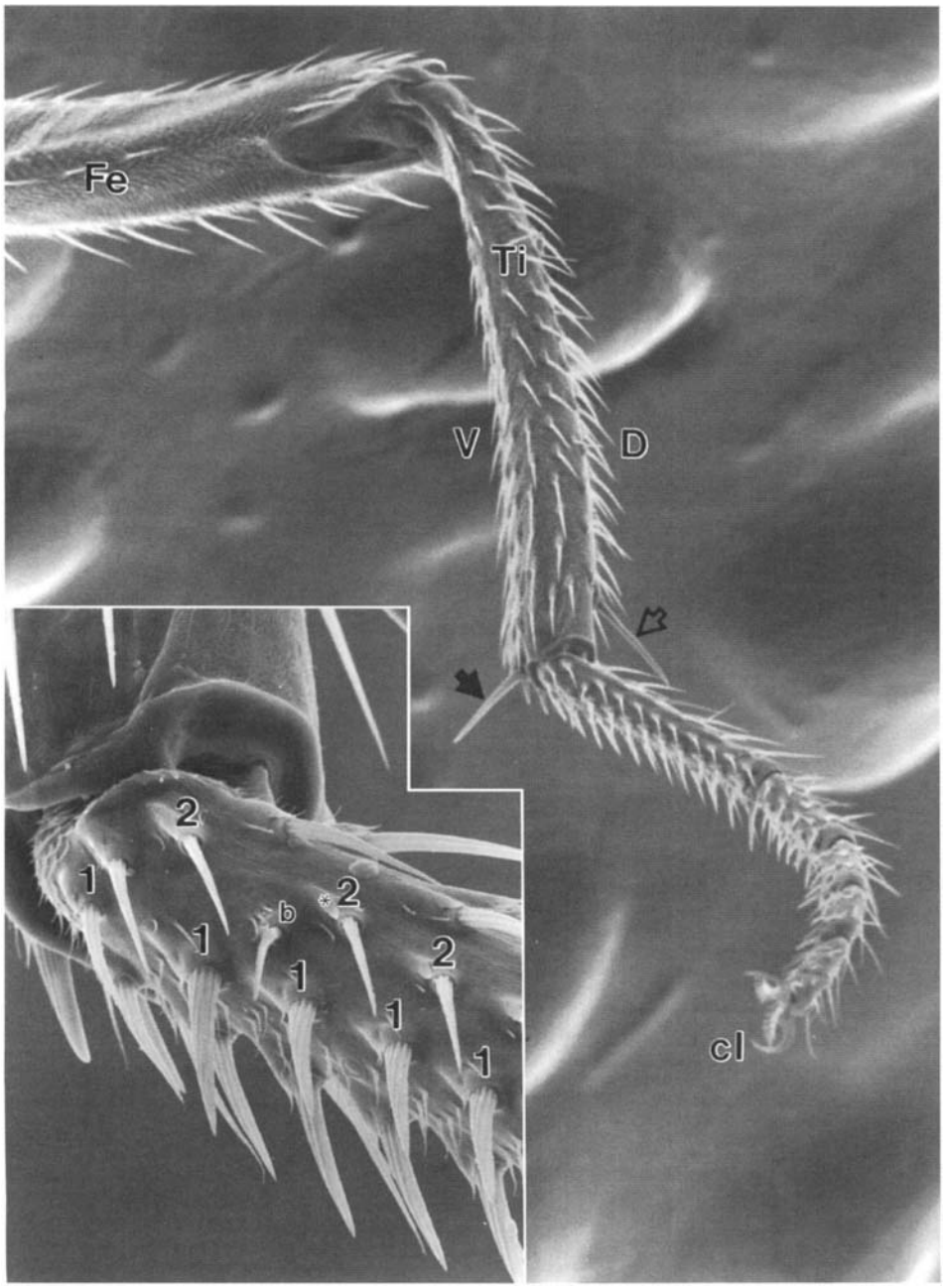

Fig. 1. Scanning electron micrograph of a right second leg of a wild-type *Drosophila* adult. The posterior surface is face-up, with the ventral (V) and dorsal (D) surfaces to the left and right. Note how the joints bend in the D↔V plane. The most proximal two segments (coxa and trochanter) and the upper part of the femur (Fe) have been cropped. The two major leg segments (whose joint functions like the human knee) bear names analogous to human leg bones – 'femur' and 'tibia' (Ti) – with the distalmost five small segments likewise termed the 'tarsus'. The tarsus bears two claws (cl) at its tip. The whole leg is 2 mm long and has about 470 bristles. Useful landmarks for identifying V-type or D-type differentiation in abnormal legs are two large bristles at or near the distal end of the tibia: the V-side 'apical' bristle (solid arrow) and the D-side 'pre-apical' bristle (unfilled arrow). Inset: Magnified view of the tibia-tarsal joint and the proximal part of the first tarsal segment (the 'basitarsus'). Most tarsal bristles have a triangular 'bract' (*) on the proximal side of their socket; 'bractless' bristles (b) have tiny hairs instead. Numbers above sockets mark bristles belonging to rows 1 and 2. (For a map, cf. Fig. 3a.) Note how much thicker the row-1 bristles appear (fluting of the shaft is also more evident) compared with row-2 bristles. Bristle thickness is a useful clue for V identity because the bristles of the other ventral row (row 8) resemble those of row 1, whereas bristles in the remaining rows are thin like row 2. Tarsal bristles range in length from 35 to 70 μm, depending upon the row[85]. Indeed, an intriguing mystery of the pattern is why there are D↔V gradients in bristle length and spacing[86], since neither gradient has any obvious adaptive function. The gradient in bristle spacing is partially visible here in the larger inter-bristle intervals of row 2 *versus* row 1.

from the symmetry plane of the tarsal bristle pattern (Fig. 3a)[21,22]. *A priori* it was natural to expect that a bilaterally symmetric pattern like the fly tarsus (or the human face) would employ a reference axis coincident with its symmetry plane. This 'Noncongruence Mystery' may now be solved, since it appears that the true reference axis is not the boundary itself but a zone slightly anterior to it – a zone that evidently does coincide with the D↔V plane of symmetry.

Importance of the *wg-dpp* border zone

By 1991 the harvest of insights into embryonic body segmentation was beginning to furnish clues as to how some of these same genes might also participate in building adult organs. Based upon the then-known patterns of postembryonic expression (or nonexpression) of the three classes of body segmentation genes, Wilkins and Gubb[23] reasoned that genes in the segment-polarity class must be instrumental in assigning positional information in discs. Much recent work has indeed implicated two segment-polarity genes (*hedgehog* and *wingless*) as key players in disc patterning, along with a third gene (*decapentaplegic*) that is not a member of the segmentation gene hierarchy. The evidence comes from *in situ* gene expression, artificially driven ectopic gene expression and mutant phenotypes.

Mutations in two genes cause dramatic 'deficiency-duplication' phenotypes in which either the ventral or dorsal portion of the leg is missing and replaced by a mirror-image copy of the remaining portion[24]. A pupal-lethal allele of *wingless* (wg^{CX3}) removes the V side, causing a double-dorsal (D/D) phenotype (imagine your hand having two backs and no palm), and adult-viable mutations in *decapentaplegic* (*dpp*) remove the D side, causing a double-ventral (V/V) phenotype (as if your hand had two palms and no back); null alleles behave similarly in genetic mosaics (L. I. Held and M. A. Heup, unpublished observations). The mirror-symmetry planes in these 'Janus' phenotypes are virtually identical, but in contrast to the semicircles defined by the straight D↔V line, the crooked A↔P line (Fig. 5a) does not bisect the disc into equal V and D halves: the V (*wg*-dependent) domain is appreciably smaller than the D (*dpp*-dependent) one.

The *wg* and *dpp* genes are transcribed in only a small portion of their respective V (135°) and D (225°) phenotypic domains (Fig. 4c) – *wg* in a sector that overlaps the V midline[25] and *dpp* in two opposite sectors that together form a stripe along the entire D↔V line[26]. (As discussed below, the transcription of *dpp* ventrally is problematic given the dorsal focus of its mutant phenotypes, but its ventral tran-

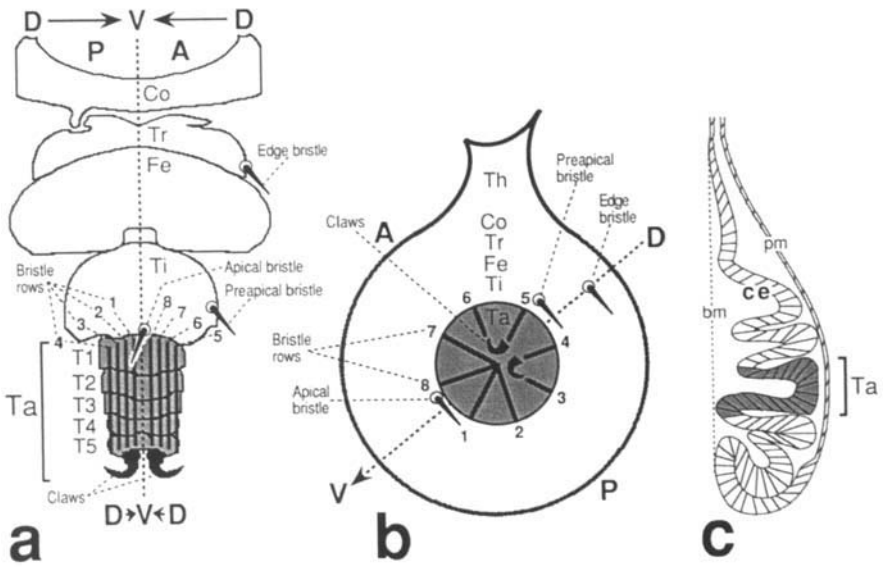

Fig. 2. Anatomy of the fly leg and organization of the leg disc. The proximal (top) and distal (bottom) ends of the adult leg (a) come respectively from the periphery and center of the leg imaginal disc (b, c). The tarsal region is shaded in all panels. (a) Schematic diagram ('pelt') of a left leg of a *Drosophila* adult. The leg is drawn as if slit along its dorsal (D) midline, pried open, and laid flat so that the entire surface is visible. The ventral (V) midline divides the map into roughly symmetrical anterior (A) and posterior (P) halves. The leg has nine segments: coxa (Co), trochanter (Tr), femur (Fe), tibia (Ti) and tarsal (Ta) segments T1 (the 'basitarsus') through T5. Vertical (proximal-distal) dimensions have here been compressed relative to horizontal. Segment shapes are similar for all six legs except for the coxa; the coxa sketched here is that of a first leg. The edge, apical and preapical bristles are useful for identifying D *versus* V territories in mutant legs, as are the various types of tarsal bristles (Fig. 3a). Longitudinal rows of tarsal bristles are denoted by thick vertical lines (cf. radial spokes in b). First and third legs also have transverse rows of bristles (not shown). (b) Fate map of a left leg disc of a mature larva, after Schubiger[87] (greatly simplified). Topologically, this (circular) map is a flat projection of the same (conical) leg surface that was fileted (tip to base) in a. Leg segments are nested as concentric circles (only the tarsal boundary is shown). The stalk (top) forms thoracic (Th) cuticle that is not part of the leg proper. The D↔V arrow refers to the future D↔V axis of the leg, not the orientation of the disc inside the larva. (NB: some authors draw the D↔V axis as a vertical line based upon the *engrailed* expression domain, whose edge runs diagonally across the center of the disc but bends vertically as it approaches the stalk[19,20]. Because this boundary is crooked, any straight line – no matter what its tilt – can only afford an approximation.) Positions of bristle rows are estimated. The claws have a slightly eccentric location in the map and behave like dorsal structures in mutant phenotypes[24]. (c) Semidiagrammatic drawing of a saggital section (essentially a side view of the disc in b) of a leg disc from a mature larva, after Poodry and Schneiderman[17]. The disc (about 10^4 cells in total) has two faces, both of which are one cell deep. Cuticular structures only come from the columnar epithelium (ce), which is thick, corrugated and underlain with a (noncellular) basement membrane (bm). The convolutions of this surface can confound efforts to determine the shapes of gene-expression domains in photographs. On the other side is a thin peripodial membrane (pm), which peels away when the leg segments telescope out during evagination[6,17]. The disc epithelium is contiguous with the larval epidermis (not shown). Lines spanning the monolayer represent cell boundaries (cell widths exaggerated). The tarsal portion of the epithelium (shaded) may be relatively larger than depicted here[6].

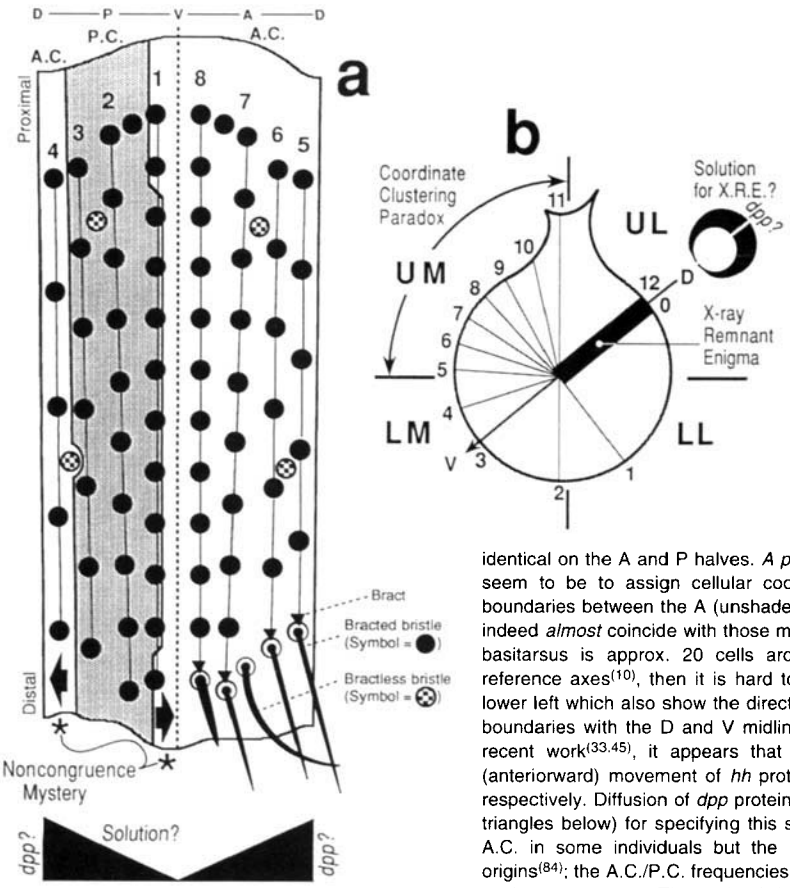

Fig. 3. Puzzling phenomena associated with *Drosophila* leg development. (a) The 'Noncongruence Mystery'. Bristle pattern of the basitarsal segment of a left second leg – essentially a magnified version of the same segment from Fig. 2a (proximal at the top, V midline centermost) except that the proximal-distal dimension is not compressed. Bristles are symbolized by circles; a few (lower right) are shown as they actually appear. The number of bristles in each row varies slightly among individuals. All but five of the approx. 80 bristles bear a 'bract' (noninnervated hair) above their socket (function unknown). Bracted (mechanosensory, straight shaft) bristles are aligned in rows (vertical lines), whereas bractless (dually chemo- and mechanosensory, curved shaft) bristles reside at characteristic inter-row sites. The eight rows are numbered as per original nomenclature[15]. (NB: in two recent articles[52,59] the nomenclature is backwards, with V rows mislabeled as 4 and 5 and D rows as 1 and 8.) The pattern has a striking bilateral symmetry about the D↔V plane: with increasing distance from the V midline, bristle rows exhibit larger bristle intervals[86] and greater bristle lengths[85], and bractless bristle positions are nearly identical on the A and P halves. *A priori* the simplest way to specify this symmetric pattern would seem to be to assign cellular coordinates relative to the D and V midlines. Strangely, the boundaries between the A (unshaded) and P (shaded) cell-lineage compartments (A.C., P.C.) do indeed *almost* coincide with those midlines[21,22], missing them by only a few cell diameters. (The basitarsus is approx. 20 cells around[86].) If compartment boundaries are supposed to be reference axes[10], then it is hard to understand the noncongruence (signified by arrows in the lower left which also show the direction of spread of the *hh* protein – cf. Fig. 4b) of the A.C./P.C. boundaries with the D and V midlines. This mystery may now be solved because, based upon recent work[33,45], it appears that the A.C./P.C. boundary is only a stepping stone for the (anteriorward) movement of *hh* protein to the D and V midlines where it activates *dpp* and *wg* respectively. Diffusion of *dpp* protein from the D midline could erect mirror-image gradients (black triangles below) for specifying this symmetrical pattern. Individual bristles of row 1 reside in the A.C. in some individuals but the P.C. in others[21,22], reflecting a slight variability in bristle origins[84]; the A.C./P.C. frequencies for each bristle are denoted by how much of its symbol lies in each compartment. The path of the A.C./P.C. boundary through the background epidermis is unknown. (b) Two other long-standing riddles of leg development: the 'Coordinate Clustering Paradox' and the 'X-ray Remnant Enigma'. Peripheral (clockface) numbers denote angular coordinates[60], not bristle rows, and the '12/0' meridian is placed at the D midline for reasons discussed in the legend of Fig. 5c. Among the four quadrants of the disc (UM, upper medial; UL, upper lateral; LM, lower medial; LL, lower lateral; named as per the disc's orientation inside the larva), only the UM quadrant can regenerate the remainder of the disc[12]. According to the Polar Coordinate Model, a fragment should only have this ability if it contains more than half of the angular positional values, implying that coordinates must be clustered in the UM quadrant[7]. Although this *ad hoc* assumption explains UM regeneration, it poses a new dilemma. The model attributes the determinate growth of discs (their ability to intrinsically stop growing at a definite size) to cells intercalating new neighbors (i.e. proliferating) until all 'discontinuities' are eliminated (i.e. all coordinates are assigned, like people filling seats in a stadium)[88]. Why then should cells in one half of the coordinate system (the UM quadrant) stop growing when their coordinate density is so much greater than in the other half[8]? This paradox has not yet been resolved. The 'X-ray Remnant Enigma' refers to duplicated legs that develop after X-raying young larvae[34] or after the induction of cell death in heat-sensitive mutants[35-37]. The affected legs are missing varying parts of the ventral and lateral circumference, with structures in the dorsal remnant being duplicated as mirror-images. Why this 'gradient of developmental capacity'[89] should run along the D↔V axis, rather than along a line bisecting the UM quadrant (where regenerative capacity is maximal) is perplexing. Since the D midline is where the *dpp* gene is strongly expressed, this midline could be a center for growth-controlling gradients, and *dpp* mutants do show cell death[70] and pattern deficiencies[24] consistent with a trophic (mitogenic) function. (However, based upon past theory, duplicative remnants should come from the low end of a gradient[34], which for *dpp* would be the V midline.) Note: the geometry of the 'curved' gradients (depicted at right as curved black triangles wrapped around a circular disc; cf. Fig. 5c) is identical to the apparently linear gradients shown in (a). (See Fig. 2 for the topological relationship of disc *versus* leg.) Thus, the idea of mirror-image, curved *dpp* gradients may help explain both the Noncongruence Mystery and the X-ray Remnant Enigma.

scription is reduced and may be nonfunctional.) Such a discrepancy between a small transcription area and a large domain of influence would be expected if the gene products were functioning as diffusible morphogens. Consistent with this notion, *wg* and *dpp* are both members of growth factor families (*Wnt* and TGF-β respectively)[27,28] and diffusion of both proteins has been demonstrated in the embryo[29,30]. Further, the *wg-dpp* transcription zone is ideally situated to serve as a reference axis since it coincides (at least approximately) with the leg's D↔V plane of symmetry. In the simplest imaginable model (Fig. 5a), the *wg* and *dpp* gene products could form a concentration gradient in each (A and P) half of the disc, and these mirror-image gradients could designate every cell's distance from the A/P boundary[10,31-33].

The importance of the D↔V axis, as revealed by *wg* and *dpp*, chimes with a peculiar old observation: the remnants of legs that develop after X-irradiation of young larvae[34], or after exposure of heat-sensitive cell-death mutants to peri-

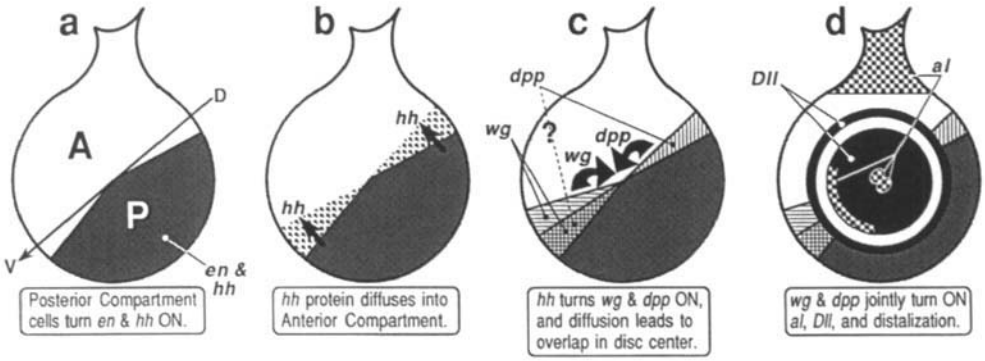

Fig. 4. (a-d) Cascade of gene actions supposedly involved in the patterning of the leg disc. Gene abbreviations: *en* (*engrailed*), *hh* (*hedgehog*), *wg* (*wingless*), *dpp* (*decapentaplegic*), *al* (*aristaless*), *Dll* (*Distal-less*). Fill-pattern shadings designate areas where the respective genes are transcribed during late third instar. (In early third instar *dpp* is apparently expressed only ventrally[26] – a problem for all of the models in Fig. 5.) These four diagrams represent a causal chain of interactions, not necessarily a temporal sequence of discrete events. That is, each gene may sustain the downstream gene's activity throughout much of development. In this 'signal relay' scenario, the A (unshaded) *versus* P (shaded) compartmental identities of cells (a) set the stage for the seepage of a posterior-specific diffusible signal (*hh*) into a border zone (b), where it induces transcription of (c) two new diffusible signals (*wg* ON ventrally; *dpp* ON all along the zone but perhaps nonfunctional ventrally), one or both of which leak out of their respective sectors to overlap in the center of the disc, where they cooperate (d) to turn ON the homeobox genes *al* and *Dll*, which may finally (not shown, cf. Fig. 5b) trigger a radially diffusible morphogen that specifies radial distances. 'Distalization' is the formation of distal leg structures (see text). Oddly (given the above scenario), mature discs manifest slight overlaps (of several cell diameters) between the *dpp* and *en* domains and between the *wg* and *en* domains[44,52], though the *dpp-en* boundary is sharp during the early third instar[44]. The diffusion range of the *hh* and *wg* proteins in the leg disc is several cell diameters[44,51]; the diffusion range for *dpp* protein is unknown. A causal relationship between *en* and *hh* (*en* activates *hh*) has recently been documented in leg discs[74]. For the sake of clarity in (d), underlying areas of gene expression (*en, hh, wg, dpp*) have not been shaded within the *Dll* domain. The size of the *wg* sector in some published pictures is considerably larger, and the *dpp*-expressing zone may look like either two tandem sectors (as here) or a single disc-spanning stripe. In (a) (not shown) *cubitus-interruptus* is expressed in the A compartment[20].

ods of high temperature[35-37], are deficient for ventral structures and duplicated for dorsal ones, much like the D/D duplicated legs of wg^{CX3} except that the degree of V loss and D duplication varies along the whole spectrum rather than showing a single (relatively stable) mirror-symmetry plane. Thus, these remnants always possess D-midline structures (Fig. 3b). An early hint about the V-midline's role in axial patterning was the finding that triplicated legs lacking V structures in their extra branches are also incomplete distally, whereas those that contain them are complete[38].

Meinhardt's Boundary Model

Because the disc epithelium is two-dimensional, the conjecture that the D↔V line functions as an 'x' axis begs the question: is there a perpendicular 'y' axis that could provide the second coordinate for a Cartesian coordinate system? The A↔P Janus-mutant symmetry line is the right place for a second axis, but no genes have yet been found that are expressed along it in a manner analogous to *wg* and *dpp*. Based upon cell-lineage evidence that a D/V boundary subdivides the leg's A compartment into D and V subcompartments[18] (but cf. ref. 21), the theoretician Hans Meinhardt formulated in 1980 what has since been dubbed his 'Boundary Model'[14]. (A similar model was contemporaneously advanced by Deak[39], and the basic idea of creating border zones *de novo* from adjacent territories is traceable at least back to Weiss[40].) He envisaged that three (or more) compartments cooperate to cause the production of a specific morphogen at their point of intersection (the center of the disc), and that the conical concentration gradient formed by the diffusion of this morphogen would specify a radial coordinate for all cells in the disc (Fig. 5b). The radial coordinate was also a key component of the Polar Coordinate Model, but unlike that model the Boundary Model did not provide for angular subdivisions any finer than the three large sectors themselves. Geometrically it is not possible to uniquely specify cellular positions in a plane using the combination of Meinhardt's radial (polar) coordinate with the *wg-dpp* zone's linear (Cartesian) coordinate, so these two partial coordinate systems cannot be reconciled in any obvious way (but see below).

To explain how two or more compartments might cooperate to activate particular genes along their common boundaries, Meinhardt suggested that *'each compartment may be responsible for a particular step in the synthesis of the morphogen or ... may produce a diffusible cofactor which is required for morphogen production'*[14]. If a cofactor that is diffusible over short distances traverses the boundary of its own compartment to interact with neighboring cells of an adjacent compartment, he reasoned, then a unique product could be made in the zone of overlap. This 'leaky border' scenario may in fact explain how the *wg-dpp* transcription zone arises (Fig. 4). Cells of the P compartment express the segment-polarity genes *engrailed* (*en*) and *hedgehog* (*hh*)[19,41]. Whereas *en* encodes a (nondiffusible) homeodomain-containing transcription factor[42], *hh* encodes a transmembrane protein that is cleaved autoproteolytically to become a diffusible product[43]. The depth of the *hh* protein's penetration into the A compartment (Fig. 4b) has been estimated by immunological probes to be a few cell diameters[44] – possibly sufficient to span the breadth of the *wg-dpp* zone[45], though it does seem odd that the *wg* sector should be so much broader than the *dpp* stripe. A causal link between *hh* and the turning ON of *wg* and *dpp* in this zone

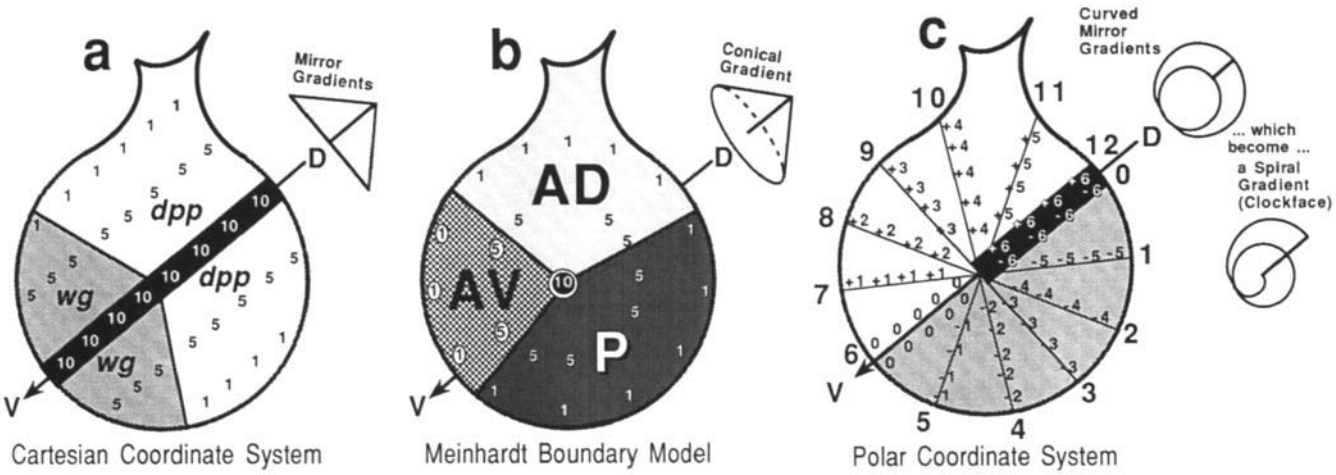

Fig. 5. Theoretical models for the specification of positional information in the leg disc. Numbers are hypothetical concentrations of the presumed morphogens [+ and – signs in (c) denote activator and repressor influences], and the small drawings to the upper right in each case depict the geometry of the morphogen gradients across the surface of the disc. (a) Cartesian Coordinate System. The *wg-dpp* zone (black stripe along the D↔V line) could function as an 'x' axis to specify one coordinate of cellular positions, though no candidate gene(s) is known that could perform a similar function for the other 'y' axis (if it exists)[31]. Numbers are concentrations of either *dpp* or *wg*, assuming lateral diffusion[32,33,45], though recent evidence argues against *wg* as a morphogen (see text). The double gradients, centered on the A/P boundary, are schematized in miniature at right. No genes are known that are expressed in stripes parallel to the *wg-dpp* zone – as might be expected if cells responded to different thresholds of the morphogen signal – nor would such stripes make sense geometrically since they would form parabolas on the conical leg surface. Shaded and unshaded sectors designate the inferred domains of influence for the *wg* and *dpp* products respectively, based upon mutant phenotypes[24], though Class-5 *dpp* mutations delete the entire tarsus[46]. The *wg*- and *dpp*-dependent sectors are separated by a crooked 'A↔P' line, which is a line of symmetry in the deficient-duplicated legs of both wg^{CX3} (D/D) and dpp^{d6} (V/V), but not for the wild-type case (D/V) shown here. (b) The Boundary Model of Hans Meinhardt[14,55]. The leg disc is known to be partitioned into two compartments (A and P), and there is weak evidence[18] that cell lineage within the A compartment is further restricted, delineating two (dorsal = AD, ventral = AV) quadrants. These three regions are assumed to cooperate (*via* overlap of diffusible, compartment-specific signals) to create (at the point of intersection of their boundaries) a morphogen molecule that diffuses throughout the disc and informs every cell of its distance from the center of the disc – in effect giving the disc a radial coordinate. The conical concentration gradient, depicted in miniature at right, would have its peak at the center of the disc. The borders (AV/AD, AD/P, P/AV) could also function as reference axes (encoding either Cartesian or angular values) *via* border-specific morphogens (not shown)[56]. (c) Spiral Gradient Model, designed as a novel way of creating the angular coordinate for a polar coordinate system. [The radial coordinate could still be generated as in (b).] The morphogen is assumed to be *dpp* protein, which diffuses *around* the disc [rather than laterally as in (a)] from its D-midline source. The numbers inside the disc indicate hypothetical *dpp* concentrations. The + and – signs denote stimulatory (A half) *versus* inhibitory (P half) effects of the *dpp* protein on an unidentified 'clockface' molecule, analogous to activin-*versus*-inhibin (homo-*versus*-heterodimer) effects that characterize other members of the TGF-β family. The A *versus* P differences in cell behavior could be dictated by genes like *en*. In the cartoons to the right, *dpp* values are plotted as radial distance from the perimeter of a circle that signifies the disc. The absolute value of *dpp* concentration yields two curved gradients (upper drawing). Assigning negative signs to *dpp* numbers in the P half (lower drawing) is mathematically tantamount to pushing the P gradient into the interior – resulting in a spiral, whence the model's name. This spiral can be converted to the canonical clockface of the Polar Coordinate Model by assuming that the nondiffusible 'clockface' molecule affected by *dpp* has a baseline concentration (in the absence of *dpp*) of 6 units everywhere. Its final concentration (computed by adding 6 to the *dpp* values) would be the numbers shown around the disc – i.e. a clockface. Although all three models invoke a diffusion mechanism, they can – in principle (not shown) – be reformulated as cellular automata where signals are instead propagated by cell-surface interactions. None of them explains the Coordinate Clustering Paradox (Fig. 3b).

(Fig. 4c) is demonstrable by 'ubiquitously' (throughout the disc) expressing *hh* (cf. Blair[13] for an explanation of the *flp*-out and other techniques), which leads to an anterior expansion of *wg* and *dpp* expression that fills the AV and AD quadrants respectively (Fig. 5b)[45,46], though *dpp* expression is also detectable at a low level in the AV quadrant[43,45]. These complementary domains, which are thus defined by a hidden 'competence' to express *wg* or *dpp*, buttress Meinhardt's assumption (based on weak cell-lineage evidence) of a second (AV/AD) compartment boundary in the leg disc.

This same leaky-border mechanism also seems to be used by *wg* and *dpp* to activate combinatorially the two homeobox genes *Distal-less* (*Dll*; a.k.a. *Brista*) and *aristaless* (*al*). Like *wg* and *dpp*, *Dll* is expressed throughout leg disc development. Indeed, it is first detectable when the leg discs arise at the intersection of perpendicular *wg* and *dpp* stripes along the D↔V and A↔P axes of the embryo, suggesting that *wg* and *dpp* may cooperate to turn *Dll* ON wherever they overlap[47,48]. In mature discs *Dll* is expressed centrally (throughout the tarsus and distal tibia) and in an outer ring (proximal femur and part of trochanter; Fig. 4d)[3]. The *Dll* protein probably helps specify or record positional information along the leg's proximal-distal axis because (1) in the absence of *Dll* function, only the base of the leg develops and (2) hypomorphic *Dll* alleles can be ordered in a series wherein varying amounts of distal leg material are missing, but *Dll* itself is probably not a direct effector of the P↔D axis since its concentration is not graded[49]. The main evidence that *Dll* is turned ON by the combined action of *wg* and *dpp* comes from discs where *hh* is ubiquitously expressed (as discussed above). In this case, a wide stripe of *Dll* expression straddles the AV/AD interface between the

enlarged *wg*-expressing and *dpp*-expressing domains[46]. Similar results had previously led to the idea that the *al* gene is governed by such a combinatorial rule – *viz.* 'IF *wg* AND *dpp* are present, THEN turn ON'. Like *Dll*, *al* is expressed in the center of the disc but in a much smaller area, confined to the claws (Fig. 4d)[50]. Also like *Dll*, there are non-central areas of *al* expression as well (which are presumably controlled by genes other than *wg* and *dpp*): a small arc of cells in the ventrodistal tibia and a large area in the dorsal coxa and pleural thorax. Unlike *Dll*, however, there are no loss-of-function alleles that could clarify the gene's function. Ubiquitous expression of *wg* causes *al*'s central spot to expand into a stripe along the D midline where *dpp* expression is maximal[50], implying a '*wg* + *dpp* = ON' combinatorial control, though such expansion does not necessarily lead to extra claws[51]. 'Proof' of the causal dependence of *al* upon *wg* and *dpp* came from an experiment in which *wg*-expressing clones were induced at random locations in the disc. Only when these clones 'hit' the *dpp*-expressing D midline was a secondary spot of *al* formed[50].

This kind of *wg* 'shotgun' experiment was first performed by Struhl and Basler (inventors of the *flp*-out strategy) who noticed that dorsally located *wg* clones can induce branched-leg duplications[52]. They interpreted the extra distal branches to mean that *wg* not only acts along the D↔V axis (where it is presumed to be a morphogen) but along the proximal-distal axis as well – a conclusion foreshadowed by the ability of *wg* mutations to cause branched legs (in addition to causing internally deficient-and-duplicated D/D legs)[53]. Because the branches also arise only along the D midline, the ability of a disc to grow a distal tip ('distalization') may also (like *Dll* and *al* expression) depend upon the combined expression of *wg* and *dpp*[50]. Distalization had formerly been explained by the Polar Coordinate Model as the outcome of cell-cell interactions, without regard to the D midline or any other unique line or point around the circumference[54].

The foregoing discussion does not adequately convey the explanatory power of the Boundary Model, which can also account for (1) the inception of imaginal disc primordia within the embryonic ectoderm at the borders of parasegment domains; and (2) the tendency for distally complete leg triplications to arise from the V side of the disc, both of which phenomena are more problematic for the Polar Coordinate Model. For a fuller treatment of the Boundary Model, see refs 14, 55 and 56.

To summarize, a new conceptual framework is emerging which challenges some tenets of the Polar Coordinate Model[33,45]. The would-be new paradigm is a variant of Meinhardt's Boundary Model, wherein the leg disc is divided into three compartments: AV, AD and P (Fig. 5b). Cells in the P compartment synthesize a diffusible signaling molecule (*hh*), which spreads several cell diameters anteriorly into a border zone that coincides with the D↔V line (Fig. 4b). There, the first signal induces two secondary signals – one in the AV quadrant (*wg*) and one in the AD quadrant (*dpp*) (Fig. 4c). Diffusion of these molecules across the AV/AD boundary causes them to overlap in the center of the disc where they cooperate to turn ON two genes (*Dll* and *al*; Fig. 4d) whose nondiffusible products (1) encode distal structures (tarsus and claws); (2) somehow (perhaps *via* a cone-shaped gradient of a diffusible morphogen) specify the proximal-distal coordinates of cells throughout the disc; and (3) cause distalization wherever they are expressed (in conjunction with *wg* and *dpp*?). Some facts that are troublesome for this scenario include: (1) why is the central domain of *Dll* so much larger than that of *al* if both are triggered by the interaction of *wg* (which has such a small diffusion radius) and *dpp*? and (2) why doesn't enlargement of the *al* domain always lead to extra claws if this gene is associated with distalization? By far the most serious difficulties, however, concern *dpp* and *wg*.

Ambiguities of *dpp* and *wg*

Why is *dpp* transcribed ventrally as well as dorsally if it only functions dorsally (Fig. 4c)? According to the Boundary Model, an interaction between *wg* and *dpp* should occur wherever they overlap, with consequent activation of *Dll*, *al* and distalization, but the latter events clearly do not happen in the ventral sector where *wg* and *dpp* are both transcribed. Transcription of *dpp* is less ventrally than dorsally[26], so perhaps a minimum amount of *dpp* protein (attained in AD but not in AV) is needed before it can react with *wg*. Consistent with this notion, overexpression of *dpp* throughout the disc can activate *Dll* in the *wg* sector (though data on distal outgrowths are scant)[46]. Unfortunately, we do not know the distribution of *dpp* protein (as opposed to *dpp* mRNA) in wild-type leg discs (not to mention experimentally altered discs) – a technical limitation due to difficulties in obtaining antibodies suitable for identifying *dpp* protein *in situ* in imaginal discs (M. Hoffmann, personal communication). Such information would not only settle whether *dpp* is translated ventrally, but could also reveal whether the protein concentration is graded across the disc and, if so, whether the gradients are linear or curved (see below). Interestingly, in the embryo where *dpp* is known to function as a dorsal morphogen, there is indeed a post-transcriptional[57] (and possibly a post-translational[58]) repression of its function ventrally (mediated by the *short gastrulation* gene).

wg protein is not detectable at distances greater than a few cell diameters beyond the anteroventral sector where *wg* is transcribed[51] – an observation which poses a problem for any model that depends upon long-range (≥10 cell diameters) *wg* diffusion. The idea that *wg* protein might function as a (long-range) morphogen to establish (curved) mirror-image gradients about the V midline was proposed by Struhl and Basler, based upon their finding that *wg*-expressing clones in the D (*dpp*) region can recruit neighboring wild-type

cells to form extra ventrolateral pattern elements[52]. Such a nonautonomous 'organizing' ability would indeed be expected for a diffusible gradient signal, but the same nonautonomy was later found to characterize D clones that lack *shaggy/zeste white 3*, a non-diffusible (negatively regulated) protein kinase in the *wg*-signaling pathway[59]. An alternative explanation is that the D-type background cells adjacent to the V-type (wg^+ or $sgg/zw3^-$) clone cells could be intercalating new lateral-type cells at the interface *via* the shortest-route rule of the Polar Coordinate Model[13,60]. The ability of dorsal wg^+ and $sgg/zw3^-$ clones to 'induce' distal outgrowths is likewise explicable by the distalization rule of the same model[53,54] (though the ventralizing and distalizing effects are separable[51,59]). Put more generally, misexpression of *any* 'regional-identity' gene in a diametrically opposite part of a polar coordinate system (e.g. *hh* at an extremely anterior location) should cause these sorts of outcomes[13].

Further evidence against a morphogen role for *wg* comes from discs where *wg* is expressed at high levels nearly ubiquitously[61]. If *wg* is a morphogen, V-type pattern elements should develop over a wider area, but this does not happen: only ventrolateral fates are augmented[51] (cf. ref. 59). Denying *wg* a morphogen role would not impact the Boundary Model (where *wg* must merely interact with *dpp* over a minimal distance – perhaps even adjacent cells – at the center of the disc), though it would preclude a Cartesian model with *wg* and *dpp* gradients flanking the A/P boundary (Fig. 5a). We would then still be left with the question of how (if *wg* protein neither diffuses far nor specifies a large range of ventrolateral fates) the domain of *wg* influence (~135° sector; Fig. 5a) can be so much broader than the domain of *wg* transcription (~30° sector; Fig. 4c)[62]. Either *wg* protein can have effects on cell identity at concentration levels below detection[63], or it triggers a lateral cascade of domino-like inductions[52,64]. Debating whether a gene functions quantitatively *versus* qualitatively may seem trivial, but it is rooted in the pivotal issue of whether embyos use analog or digital logic[65,66].

Caution must be exercised in interpreting misexpression studies because as French and Daniels have rightly stated: *'it is ... unsafe to deduce normal gene function [when] the product is forced into inappropriate cells, perhaps in the absence of proteins with which it normally interacts and the presence of others that it does not normally encounter'*[60]. With this caveat in mind, consider that dorsal ectopic expression of *wg* in the leg disc can also induce a 'blastemal' kind of undifferentiated state[51], and transdetermination of leg cells to make wing structures[67] – radical observations which imply that *wg* may 'plug into' gene circuits for dedifferentiation and disc-type identity in addition to its still-uncertain role in narrowly specifying ventral cell identities.

Rethinking *dpp* and the Cartesian/polar dichotomy

Part of the problem in trying to decipher how genes control a patterning process is that, as discussed above for *wg* and demonstrated recently for *en*[68], a gene may play multiple roles that are experimentally and conceptually difficult to disentangle[66]. Genes whose products function as 'growth factors' pose a special problem in this regard because pattern formation in epimorphic fields is so intimately linked to growth[7]. Until recently, *dpp* was thought to function primarily along the proximal-distal axis in discs because *dpp* mutations cause distally deficient appendages[23,31,69], but much of that distal loss is due to extensive cell death[70], suggesting a trophic (cell-survival) role[71] for *dpp* in addition to its patterning functions. The discovery that *Dll* and *al* depend upon the interaction of *dpp* and *wg* has redirected our attention to the dorsal half of the *dpp* stripe, and a reexamination of the legs of Class 3 hypomorphic mutants has shown that the *dpp* 'sphere of influence' is mainly dorsal, rather than distal – comprising a 225° sector centered on the D midline (Fig. 5a)[24]. The idea of *dpp* as a dorsalizing morphogen is consistent with (1) the known role of *dpp* in the embryo where D↔V *dpp* gradients specify cell fates within the dorsal 40% of the ectoderm[58,72] and (2) the inferred role of D↔V *dpp* gradients in the brain, where they apparently guide afferent retinal projections[73].

In the original Boundary Model[14], the sole morphogen was the one that generates a cone-shaped gradient to specify the radial positions of all disc cells (Fig. 5b), and although that morphogen and its gene(s) are only hypothetical, the ring-shaped expression patterns of at least half-a-dozen genes[62] (turned ON at different levels of the gradient?) are consistent with the radial gradient idea. However, the cuticular pattern of the leg has a much finer grain (about 500 bristles arranged in orderly patterns) than three large domains (AV, AD and P), so cells must receive other information about their positions[61]. In a paper published three years after he introduced his model, Meinhardt answered this criticism by asserting: *'The disc begins with a coarse circumferential determination – namely, the three sectors ... of the major compartments. The compartment borders must act as a frame for the finer circumferential subdivision ... The distance from a particular compartment border could be measured by a diffusible morphogen'*[56]. He cites Crick and Lawrence[10], who envisaged a pair of back-to-back gradients centered on the A/P compartment boundary. Their mirror-gradient idea (Fig. 5a) was partly based on the ability of the en^1 mutation to convert P structures (in the wing and leg) into their A counterparts. Subsequently, Lawrence and his collaborators have argued that the mirror-gradient morphogen is *dpp*[32,45] – a possibility that is made even more likely by the recent demise of *wg* as a morphogen candidate[74].

A 'Spiral Gradient' model

Rationale

Other authors have shown how the recent molecular evi-

dence fits Meinhardt's Boundary Model[33]. Here, an attempt is made to show that: (1) this evidence can also be accommodated by the Polar Coordinate Model; (2) the angular coordinate of a polar system could be constructed *via* a Cartesian axis; and hence (3) the superficially different Cartesian and polar systems are fundamentally compatible, as has been argued before in other contexts[75-78].

Assumptions

In Crick and Lawrence's back-to-back gradients (Fig. 5a), the morphogen diffuses freely to form triangular concentration profiles on either side of the A/P compartment boundary[10]. Whereas this geometry may suit the wing disc where the incipient wing veins run parallel to this boundary, it does not fit the leg disc where the bristle rows arise as radial spokes (Fig. 2b). Radial rows could, however, be specified as contour lines at successive levels of mirror-image gradients if the gradients were curved instead of straight (Fig. 5c). Thus, the first set of assumptions is: (1) the morphogen is the *dpp* protein; (2) although *dpp* is transcribed both dorsally and ventrally, only the D midline cells make a functional *dpp* protein; and (3) the protein is constrained to move along circular arcs, rather than straight lines. Fig. 5c shows that if a band of *dpp*-synthesizing cells along the D midline maintains the *dpp* concentration at say 6 units, then the concentration could decay to zero by the time the V midline is reached. The eight tarsal bristle rows could then be specified at *dpp* levels 5.25, 3.75, 2.25 and 0.75 on either side of the A/P boundary (not depicted), with each row adjusting its bristle spacing and bristle lengths relative to these same levels so as to yield the actual gradients observed in these variables on the adult tarsus (Fig. 3a; cf. refs 58 and 72). The final set of assumptions is aimed at converting this pair of curved gradients into a clockface of angular coordinates: (4) the *dpp* protein modulates the amount of a nondiffusible 'clockface' molecule (identity unknown); (5) in the A half of the disc, *dpp* protein stimulates clockface molecule synthesis, whereas in the P half, *dpp* protein inhibits it; and (6) the clockface molecule functions as the angular coordinate. In Fig. 5c, the opposite effects of *dpp* are denoted by plus and minus signs. If the baseline concentration of the clockface molecule (in the absence of *dpp*) is 6 units throughout the disc, and its value changes from the baseline by an amount equal to the *dpp* concentration, then the final values would be 12 units (6 + 6) just anterior to the D midline and 0 units (6 – 6) just posterior to it, with a clockface of intermediate numbers around the perimeter.

Justification of assumptions

(1) The evidence for *dpp* as a morphogen has been discussed; (2) *dpp* transcription is less ventrally than dorsally, perhaps indicating repression by other genes expressed in the V region (*wg*?[24]), so post-transcriptional repression is plausible; and (3) the leg disc epithelium has concentric circular grooves (Fig. 2c), which might constrain protein diffusion. The idea (assumptions 4-6) of a '+' *versus* '–' duality in downstream effects is natural for *dpp* because of the activin-*versus*-inhibin (homo-*versus*-heterodimer) duality that characterizes other members of its growth factor family[79], and the D↔V axis of the *Drosophila* embryo is known to employ an activator-*versus*-repressor switch for the morphogen that is made by the gene '*dorsal*'[80].

Utility

Via this mechanism, a Cartesian axis (the A/P boundary) could be used to anchor the angular coordinate of a polar system, and cells might not perceive the '0/12' line as a discontinuity because it is seamless with regard to the *dpp* variable. The clockface variable could be used for regulating growth, guiding intercalation and specifying structures that are not symmetric about the D↔V plane. Thus, disc cells might have available to them (at all times during development) two superimposed coordinate systems – a symmetrical (single-axis) Cartesian one and an asymmetrical polar one (complete with radial and circumferential variables). Alternatively, the gradients could have an early patterning function (i.e. demarcating coarse territories but leaving the details to a self-sustaining polar system that it helps to erect[13,69]; cf. ref. 81) and a later growth-promoting function. Hierarchical models of this kind may ultimately prove useful in tackling the deeper genetic mysteries of asymmetry, chirality and polarity[82].

Predictions

Tests of the 'Spiral Gradient' Model could include: (1) looking for *dpp* protein gradients[83] in the leg disc and (2) comparing the compositions of the (dimeric?) proteins in the A *versus* P halves, which should differ. Another prediction concerns the tarsal bristle rows, which come from eight stripes where the proneural gene *achaete* is expressed – stripes that are delimited by four 'interband' stripes expressing the pair-rule gene *hairy*[84]: *achaete* and/or *hairy* should have enhancers that respond to discrete ranges of *dpp* concentration.

Critique

Major flaws of the model include: (1) it implies – contrary to the 'seamless regeneration' result which led to the Polar Coordinate Model – that a special boundary (the D midline) is critical for pattern formation; (2) the gradient peak seems at the wrong end of the D↔V axis to produce dorsal X-ray remnants, which should come from the *low* end of a gradient[34]; and (3) it fails to explain the clustering of angular values in the UM quadrant (Fig. 3b). Most disturbing is the difficulty of imagining how the movement of *dpp* protein could be constrained to travel along curved pathways over dis-

tances of 100-or-so cells (with no leakage across the disc center), though similar criticisms plague the well-documented gradient of activin in *Xenopus*[83]. Even if this spiral hybrid of the Polar Coordinate and Boundary Models dies stillborn, there is every reason to expect more offspring from these theories as the genetic and molecular saga unfolds.

Origins revisited

At the beginning of this essay the question was raised about the possible origin of the arthropod leg as a miniature version of the annelid body column. The idea was dismissed because the battery of segmentation genes that partitions the body column into segments apparently does not serve a homologous function in the leg. Now, with much evidence pointing to a role for *dpp* as a dorsalizing morphogen in the leg analogous to its known role as a dorsalizing morphogen in the embryo, the possibility of at least a partial body-limb homology has resurfaced. The current explosion of molecular-genetic discoveries may therefore not only help to illuminate developmental mechanisms, but may also ultimately enable us to peer back through the mists of evolutionary time to discern how the mechanisms themselves came to be.

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Note added in proof

For a different perspective, see Campbell and Tomlinson (1995) *Development* **121**, 619-628, but note that the *dpp* antibody that they used (their Fig. 5D) manifests detectable cross-reactivity only with the amino terminus of the *dpp* protein, not with the carboxy-terminal domain that is presumed to be the secreted growth factor and morphogen (ref. 29 and M. Hoffmann, personal communication). Also, a final clarification: the spiral at the right in Fig. 5c does not imply negative *dpp* concentrations, even though one half of the spiral is inside the circle. A plot of the clockface protein's concentration would be a spiral completely external to the circle.

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