

Developmental basis for vein pattern variations in insect wings

JOSÉ F. DE CELIS* and FERNANDO J. DIAZ-BENJUMEA

Centro de Biología Molecular "Severo Ochoa", Universidad Autónoma de Madrid, Spain

ABSTRACT The venation patterns characteristics of different insect orders and of families belonging to the same order possess enormous variation in vein number, position and differentiation. Although the developmental basis of changes in vein patterns during evolution is entirely unknown, the identification of the genes and developmental processes involved in *Drosophila* vein pattern formation facilitates the elaboration of construction rules. It is thus possible to identify the likely changes which may constitute a source of pattern variation during evolution. In this review, we discuss how actual patterns of venation could be accounted for by modifications in different Pterygota of a common set of developmental operations. We argue that the individual specification of each vein and the modular structure of the regulatory regions of the key genes identified in *Drosophila* offer candidate entry points for pattern modifications affecting individual veins or interveins independently. Assuming a general conservation of the processes involved in different species, the transitions between different patterns may require few changes in the regulatory gene networks involved.

KEY WORDS: *vein formation, insect orders, diptera, developmental mechanism*

"At last the time has come when we believe that we understand the homologies of the wing-veins in so large a proportion of the orders of insects that we are able to present a hypothetical type to which the wings of all orders may be referred."

J.H. Comstock and J.G. Needman (1899)

"Archetypes" are the holy grail of evolution, because they are thought to contain the characteristics from which recent patterns can be derived through series of modifications. This implies that all organisms conforming to the same archetype have a common ancestor. It also implies that structures based on an archetype must share the same phylogenetic origin, i.e. are homologous. By knowing the archetype of a given structure and identifying the genes and developmental processes involved in its generation in a number of model species, it would be possible to unravel the genetic basis of pattern diversification during evolution. The wings of insects are in this respect in a privileged position, since it is generally accepted that wings had originated only once in the Arthropod lineage and have suffered considerable variation in shape, size and patterns of cell differentiation (Kukalova-Peck, 1978; Kristensen, 1981). The wing has also been the subject of extensive analysis in a number of species, although our knowledge of the genetic basis of wing development is mainly restricted to the fruit fly *Drosophila melanogaster* (Blair, 1995). The monophyletic origin of the wing suggests that similar genes and developmental processes to those of *Drosophila* are involved in wing formation in

a great majority of insects. Given our knowledge of the rules linking gene activity with pattern formation (developmental operations) in *Drosophila*, the main subject of this review is to discuss how actual patterns of venation can be accounted by modifications in different lineages of Pterygota of a common set of developmental operations. Although we will consider the causes of pattern variations only theoretically, the development of a number of techniques such as RNA interference, gene sequencing and gene expression analysis of the relevant genes in different species will make possible to approach the problem of wing vein pattern diversification experimentally.

Wing modifications in Pterygota

Most adult insects bear wings in the meso- and metathorax. Wings, however, can be absent as a primitive character (in Apterigota Hexapods) or as an atrophy of pre-existing organs in some Pterygota belonging to many different orders (Imms, 1964). The wings of different insects present many modifications in shape, size and patterns of cell differentiation that underlie their adaptation to a variety of functions such as locomotion, defence (fore-wings in Dermaptera, Coleoptera and Orthoptera), melanism (as in Lepidoptera) and regulation of body temperature (Imms, 1964). Secondary adaptations of either the fore- or hind-wings also occur in Strepsiptera and Diptera, respectively (Fig. 1 D,G). When the fore- and hind-wings are both membranous, they can be very similar to

*Address correspondence to: Dr. Jose F. de Celis. Centro de Biología Molecular "Severo Ochoa", Universidad Autónoma de Madrid, Madrid 28049, Spain. Tel: +34-91-397-4129. e-mail: jfdecelis@cbm.uam.es

each other in size and pattern of veins (Odonata, Neuroptera) or can differ greatly in size, shape and pattern of veins (Ephemeroptera, Hemiptera, Lepidoptera, and Hymenoptera; Fig. 1) (Imms, 1964). The systemic differences between the fore- and hind-wing pairs that characterise each order of insects most likely rely on the activity of the homeotic genes and their cascades of downstream effectors (Duncan, 1987; Akam, 1987). This is suggested by the conservation of the homeotic gene complexes in Arthropods and by the effects of *Ultrabithorax* mutations in *Drosophila* wings, where the pair of metathoracic halteres is transformed into a pair of wings (Lewis, 1978; Carroll, 1995; Averof and Akam, 1995; Cook *et al.*, 2001). Homeosis is also known in some Lepidoptera associated to the transformation of the hind wings into fore wings (Sibatani, 1980). Similarly, it is likely that the existence of wingless forms in many Pterygota insects is somehow related to the mechanisms that trigger wing formation in *Drosophila*. The development of both the wing and haltere in *Drosophila* depends on the function of the *vestigial* gene (Williams *et al.*, 1991; Kim *et al.*, 1996). This gene encodes a co-transcription factor that together with the product of *scalloped* regulates gene expression programs in both wing and haltere (de Celis, 1999). The expression of *vestigial* is restricted to the corresponding imaginal primordia, and the initiation of wing blade formation is intimately related to the mechanisms that regulate *vestigial* expression (Kim *et al.*, 1996). It is not known whether *vestigial* orthologs in other winged insects perform a similar role, or whether these putative orthologs exist at all in Apterigota insects. Many mechanisms disrupting *vestigial* expression and/or function during development are conceivable, and they could be operative in groups that produce both winged and wingless individuals such as the members of the Formicidae family (ants) as well as in other wingless Pterygota species.

The origin of the wing

It is not known when wings arose in the Arthropod lineage, although it is generally assumed a monophyletic origin for the wings based on the uniformity of wing characters (Kukalova-Peck, 1978). Fossil records of current Pterygota groups are abundant in the Carboniferous period, but earlier fossil records are almost missing. There are several theories to explain the origin of the wing (Kukalova-Peck, 1978). The "paranotal" theory suggests that wings come from lateral expansions of the thorax that later in evolution acquired a direct articulation with the tergal region and independent movement by novel musculature. However, the position of the wing anlage in insects belonging to different orders shows that this originates as a separate structure from the tergum located above the spiracle (Tower, 1903; Kukalova-Peck, 1978). The wing anlage migrates dorsally and becomes fused to the tergum as development proceeds. These observations lead to suggest that wings were primarily originated from the pleura and are serially homologous to the abdominal gill-plates of Ephemeroptera nymphs and Paleozoic Protorthoptera (Kukalova-Peck, 1978). Finally, it has been proposed that the wing results from modifications of a complex paranota constituted by elements from both pleural and tergal origin (Rasnitsyn, 1981). Embryological and developmental studies in *Drosophila* are more consistent with a pleural origin of the wing. Thus, the wings in *Drosophila* arise in close association with the leg, so both structures share the same anlage (Cohen *et al.*, 1993; Goto and Hayashi, 1997). Later during embryogenesis the

wing primordium migrates dorsally and becomes separated from that of the leg. Furthermore the expression pattern of *Artemia franciscana* orthologues to the *Drosophila* genes *apterous* and *rubbin*, which are directly related to wing development, becomes restricted to the dorsal part of multibranching appendages in some crustaceans, supporting a close relationship between the Crustacean epipodites (gill-like structure) and insect wings (Averof and Cohen, 1997).

The development of veins

The veins are the most characteristic structures of the wing, appearing as longitudinal stripes of cells that differentiate darkly pigmented cuticle and are more packed than intervein cells. Longitudinal veins typically run from the base to the apex of the wing and are distributed in species-specific two-dimensional patterns (Bier, 2000; de Celis, 2003). Veins serve to strengthen the wing and also enclose conducts in which the haemolymph can circulate and that may carry trachea and axons (Waddington, 1940). Before considering vein pattern variations, we will summarise some aspects of vein development relevant to the origin of veins. In insects with incomplete metamorphosis (hemimetabolous) the development of the wing occurs externally to the body and starts as a thickening of the hypodermis that grows dorsal and ventral sides that later become apposed, leaving in between some lacunae filled by blood (Kukalova-Peck, 1978) (Fig. 2). These lacunae are invaded by tracheal branches, named costo-radial and cubito-anal group, coming from the spiracles of two adjacent segments and entering through the base of the wing (Comstock and Needham, 1898; Kukalova-Peck, 1978). It is only after the pattern of lacunae and the invasion of the tracheal

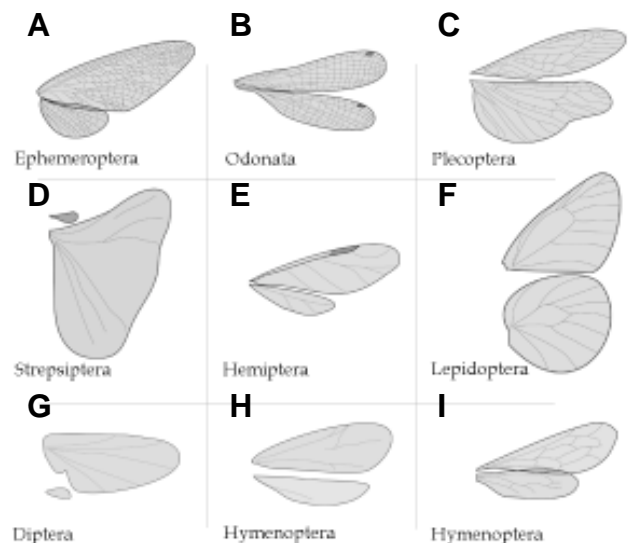


Fig. 1. Representative examples of fore- and hind wings in different orders of Insects showing their typical venation patterns. (A) Ephemeroptera (Mayflies), (B) Odonata (Dragonflies), (C) Plecoptera (Stoneflies), (D) Strepsiptera (Stylopids), (E) Hemiptera (Homoptera; Aphid), (F) Lepidoptera (Butterflies), (G) Diptera (Flies), (H) Hymenoptera (Gall Wasps), (I) Hymenoptera (Social Wasps).

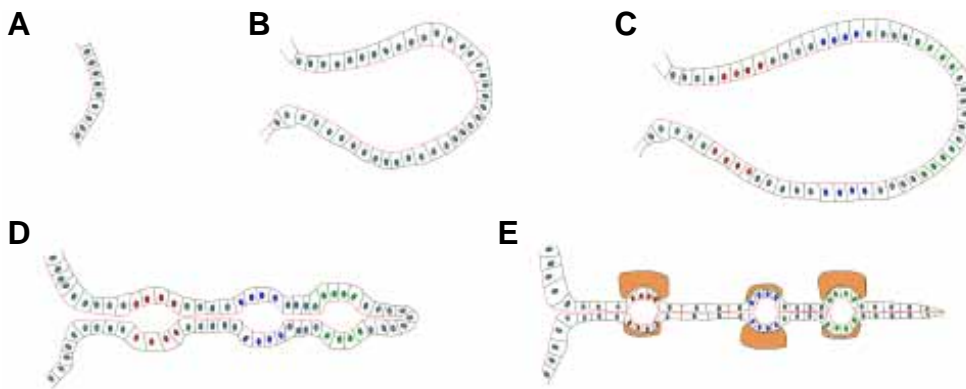
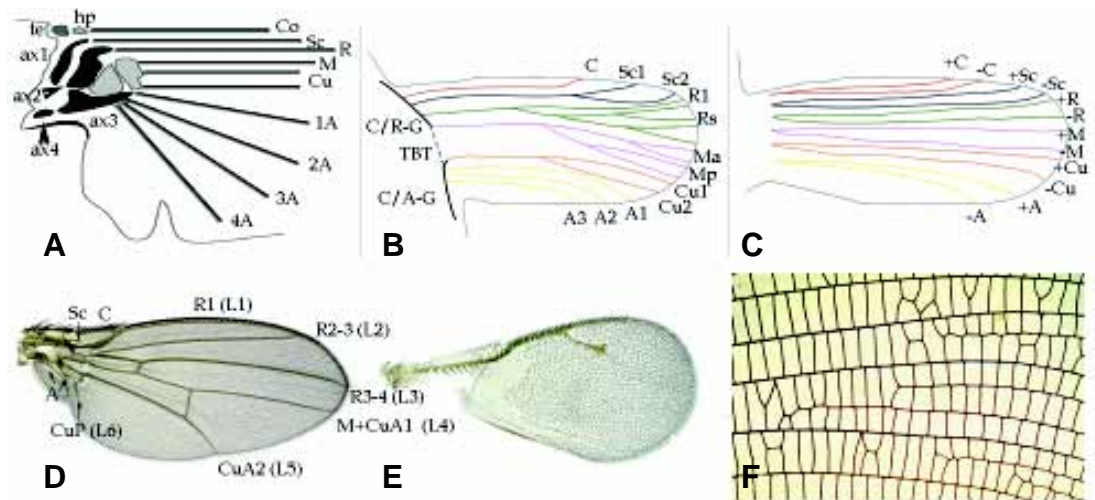


Fig. 2. Representation of the developmental steps characteristic of wing development and vein formation in Pterygota. (A) Determination of the wing anlage. **(B)** Growth of the wing primordia. **(C)** Genetic specification of veins (Coloured nuclei) and interveins (Grey nuclei). **(D)** Apposition of the dorsal and ventral wing surfaces and formation of lacunae. **(E)** Differentiation of cuticle with vein characteristics preferentially in the dorsal (above) or ventral (below) sides of the wing.

branches is established that the epidermal cells encircling the lacunae secrete the distinct cuticle that characterises the adult veins (Fig. 2). The correspondence between the pattern of both lacunae and tracheal branches with the veins in many hemimetabolous suggested a determining role for these structures in the positioning of the veins (Comstock and Needham, 1898). The development of the veins in *Drosophila* (holometabolous) shares many morphological similarities with the process described above, particularly during the pupal stage. Thus, after metamorphosis the dorsal and ventral components of the wing contact through the basal cell membranes and go through various stages of apposition-separation in which different patterns of lacunae are recognisable (Waddington, 1940; Fristrom *et al.*, 1993). As it is the case for hemimetabolous insects, the pattern of lacunae became very similar to the pattern of veins, and mutations affecting the adult veins also prevent the formation of lacunae (Waddington, 1940). This suggested that adult veins are formed after and

following the pupal lacunae (Waddington, 1940). However, in *Drosophila*, as in other insects with complete metamorphosis, the development of the wing in the larval instars occurs inside the body in an epithelial monolayer called wing imaginal disc. During the growth of the disc the dorsal and ventral halves of the future wing are adjacent to each other, and the only contact between them occurs at the future wing margin (Fig. 2). Interestingly, vein formation in *Drosophila* is initiated in the wing disc by specific programs of gene expression without any morphological manifestation (de Celis, 2003) (Fig. 2). If this were the case for other insects, vein cell specification would be determined in the growing wing epithelium by the expression of genes restricted to presumptive veins. This would be followed by the formation of lacunae due to differential adhesion in the veins and interveins between the dorsal and ventral components of the wing, and culminated by the secretion of a more pigmented and hardened cuticle by vein cells (Fig. 2).

Fig. 3. Prototypical venation patterns and extreme departures from the *Drosophila* vein pattern. (A) Contacts of the main veins in the axillary region. Abbreviations: Co, Costa; Sc, Subcosta; R, Radius; M, Medial; Cu, Cubital; 1A-4A, anal veins 1 to 4; ax1 to ax4, axillary sclerites 1 to 4; te, tegula; hp, humeral plaque. Modified from Seguy (1959). **(B)** Comstock-Newman six veins and their ramifications based on the trachea entering the wing. The meso- and metathoracic spiracles form the tracheal branches entering the mesothoracic wing, and the metathoracic and first abdominal spiracles originated the trachea entering the metathoracic one. The only exception to this pattern occurs in *Ephemeroptera* wings, where tracheation is restricted in each segment to its own spiracle, and the costo-radial and cubito-anal branches both originates from the anterior spiracle of the corresponding segment. Abbreviations: C, costa; Sc1 and Sc2, subcosta branches 1 and 2; R1, radius 1; Rs, radial sector (Radius 2 to 5); Ma and Mp, medial anterior and posterior; Cu1 and Cu2, cubitus branches 1 and 2; A1 to A3, anal veins 1 to 3; C/R-G, costo-radial branches; TBT, transverse trachea; C-A-G, cubito-anal tracheal branches. **(C)** The Kukulova-Peck (1978) proposed pattern of six pairs of veins of ancient Pterygota fossils. Plus (+) and minus (-) symbols refer to the corrugation of the vein (concave or convex). **(D)** *Drosophila melanogaster* wing showing *Drosophila* nomenclature and likely vein homologies. Modified from Garcia-Bellido and de Celis (1992) and Stark *et al.* (1999). **(E)** Veinless wing of a Hymenopterous (Superfamily Chalcidoidea). **(F)** Region of an Odonata wing showing the complex pattern of transverse veins (archedictyon).



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The structure of the ancient venation pattern

Although the vein patterns of extant insect orders is very diverse, it was recognised very early that “a uniform nomenclature of the wing veins based on homologies and, therefore, applicable to all orders, is possible” (Comstock and Needham, 1898). In this section we discuss the nature of the hypothetical wing vein pattern archetype, and the difficulties of establishing what could have been the primitive pattern of veins based on the fossil record. The origin of the most used nomenclature system for the main veins can be traced back to Redtenbacher in 1886, who recognised six veins with constant contacts with the axillary sclerites in the wing hinge (Fig. 3A). These veins are, from anterior to posterior, Costa (C), Subcosta (Sc), Radius (R), Media (M), Cubitus (Cu) and Anal (A) veins (Fig. 2). The identification of the same veins in many orders of insects suggested homology between veins, and implies that regularity in vein pattern occurred before the radiation of Pterygota insects. The nomenclature of Redtenbacher was adapted with minor modifications in the classical study of Comstock and Needham (1899), who referred vein patterns to the trachea that both precedes and prefigures the position of the veins in many insect orders (Fig. 3B). It was suggested that this pattern of six main veins constituted the ancestral one, and that other patterns can be derived either by addition or, more frequently, by reduction of particular vein branches (Comstock and Needham, 1898; Comstock and Needham, 1899). The archetype nature of this pattern was challenged based on the fossil record, and it seems clear that it does not reconstruct a primitive venation (Sharov, 1966). Thus, some of the earliest winged insects on fossil record (Protorthoptera from the Upper Carboniferous) show corrugated veins with the spaces between them filled by a dense network of transverse veins (archedictyon) (Kukalova-Peck, 1978). A full pattern of six pairs of veins is found in Paleozoic representatives of several orders and it may well be a general feature present in the ancestors of all Pterygota lineages (Kukalova-Peck, 1978). Thus, it was proposed that each of the originally identified six veins was primitively composed of two veins with opposite corrugation, one dorsal (concave) and other ventral (convex) (Fig. 3C) (Kukalova-Peck, 1978). It is not entirely clear, however, whether corrugation is an ancient character, as other paleontological evidence, the oldest known winged insect fossil (Protoptera) possesses wings without corrugation, does support the idea that corrugation is a more recent adaptation (Rasnitsyn, 1981). Irrespectively of corrugation being primitive or a derived character, in the lower orders Ephemeroptera and Odonata as well as in many fossil orders the convex or

concave condition of each vein is constant, which helps to identify vein homologies. Although these features are not so evident in higher orders (Diptera, Hymenoptera), because the wings tend to be flattened, it is still remarkable that each individual vein generally has a predominant dorsal or ventral component that is somehow analogous to the convex or concave corrugation.

The study of recent patterns with respect to an ancient prototype can be confusing, because each pattern could be considered as a mosaic of primitive and derived characters (Stark *et al.*, 1999). Furthermore, because of its complexity, it is difficult to imagine that the regular pattern of six pairs of veins (homologous throughout the Pterygota) joined by an archedictyon of transverse veins (extant in living Ephemeroptera and Odonata) constitutes the original venation pattern of a primitive wing. Despite the lack of fossil evidence previous to the Upper Carboniferous it is conceivable that regular branches with alternate corrugation evolved from a more regular pattern over a long period of time. In this hypothetical transition some branches could become reinforced over others to give rise to the six pairs of veins pattern in a common Pterygota ancestor.

Trends in venation patterns among insect orders

A remarkable characteristic of venation patterns is its diversity both among orders and within families belonging to the same order (Imms, 1964)(see Fig. 3 D-F). An exhaustive comparative analysis of insect venation patterns is beyond the scope of this review, but can be found in the work of Seguy (1959). We have selected several characteristics typical of extant orders illustrat-

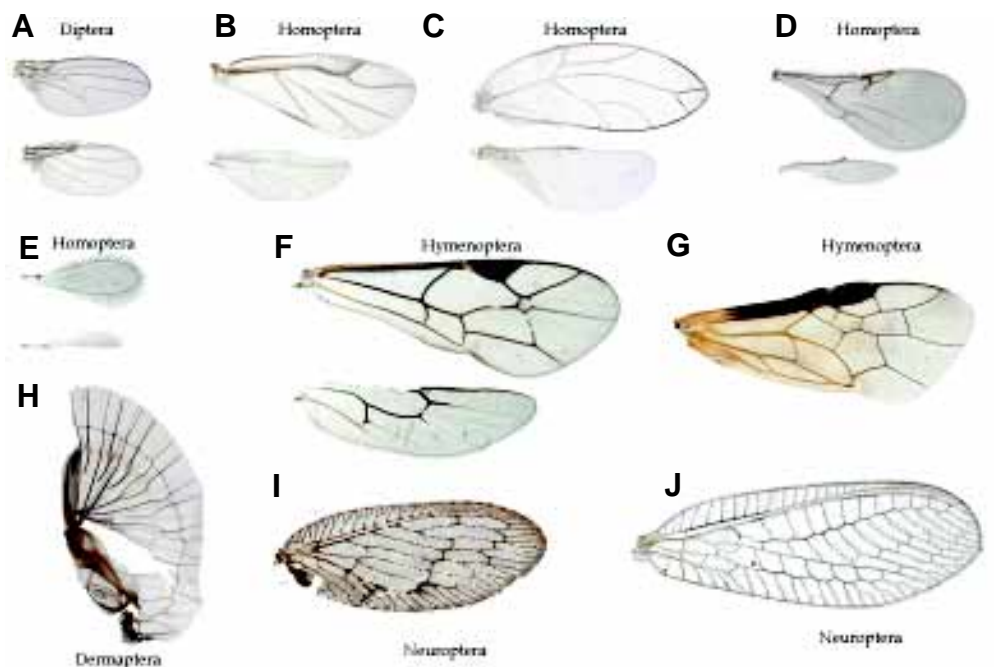


Fig. 4. Representative examples of wing morphology in several insects, illustrating trends in venation patterns. (A) *O. Diptera*, *F. Drosophilidae* (above) and *F. Phoridae* (below). (B) *O. Homoptera*, *F. Aphididae*. (C) *O. Homoptera*, *F. Psyllidae*. (D) *O. Homoptera*, *F. Aphididae*. (E) *O. Homoptera*, *F. Pteromidae*. (F) *O. Hymenoptera*, Sub Order Apocrita. (G) *O. Hymenoptera*, Sub Order Apocrita (fore-wing). (H) *O. Dermaptera*, *F. Forficulidae*. (I) *O. Neuroptera*, *F. Osmylidae*. (J) *O. Neuroptera*, *F. Chrysopidae*. Photographs in panels (A - G) are shown at the same magnifications. Panels (H - J) are shown at the same magnification and 30 times reduced compared to A-G.

ing trends underlying vein pattern variation. Determining how modifications to a common set of developmental operations may account for them is a challenge for future experimental and comparative analysis.

1) Arqueduction

Living Paleoptera of the Odonata and Ephemeroptera orders show a complex pattern of transverse veins joining adjacent branches of the longitudinal veins along their entire proximo-distal length (Fig. 3F). The complex network of transverse veins is also retained in insects belonging to the orders Dictyoptera, Orthoptera and Phasmida, as well as in many Isoptera, Homoptera, Neuroptera, Plecoptera and Tricoptera species (Seguy, 1959; Imms, 1964; Chinery, 1993). The transverse veins in these wings are not equivalent to the crossveins, which appear in very fixed positions (Comstock and Needham, 1998). Thus the elements of the arqueduction are not very precisely positioned, showing variations between left and right wings of the same animal. The recurrence of spaced transverse veins in very distant orders suggest that the arqueduction has been acquired and lost independently many times during insect evolution.

2) Vein bifurcations

In general, the main veins bifurcate at characteristic proximo-distal points, giving rise to quasi-parallel branches reaching the wing margin independently. Vein bifurcations affecting several longitudinal veins and happening many times in the course of the vein are very preponderant in wings of Neuroptera and Dictyoptera, and, to a lesser extent, are also present in many Homoptera, as well as in some Diptera and Hemiptera (Seguy, 1959; Imms, 1964; Chinery, 1993) (Fig. 4).

3) Asymmetrical development of specific branches

The ancient regular pattern of veins has suffered many modifications that in some cases are characterised by a preponderant development of some veins. The increase in the number of veins is brought about by bifurcations and development of secondary veins, but there is no increase in the number of principal veins. Some examples are the massive development of the anal veins in Dictyoptera, Plecoptera, Dermaptera and Orthoptera, where many independent anal veins fill the enlarged anal lobes, the development of costal veinlets in Neuroptera, and the formation of accessory cubital branches in Isoptera and Dictyoptera (Seguy, 1959; Imms, 1964; Chinery, 1993) (Fig. 4).

4) Reduction and elimination of specific veins

Most departures from the ancient pattern of six major veins or vein pairs arise by reduction in their numbers. In different patterns, the reductions result from the fusion of adjacent veins, which can be partial or complete, distal or proximal, or by the elimination of specific branches. Extreme cases of reduction are apparent in Embioptera, where only the radius is a prominent vein; Coleoptera with the presence of a thickened R1 and the

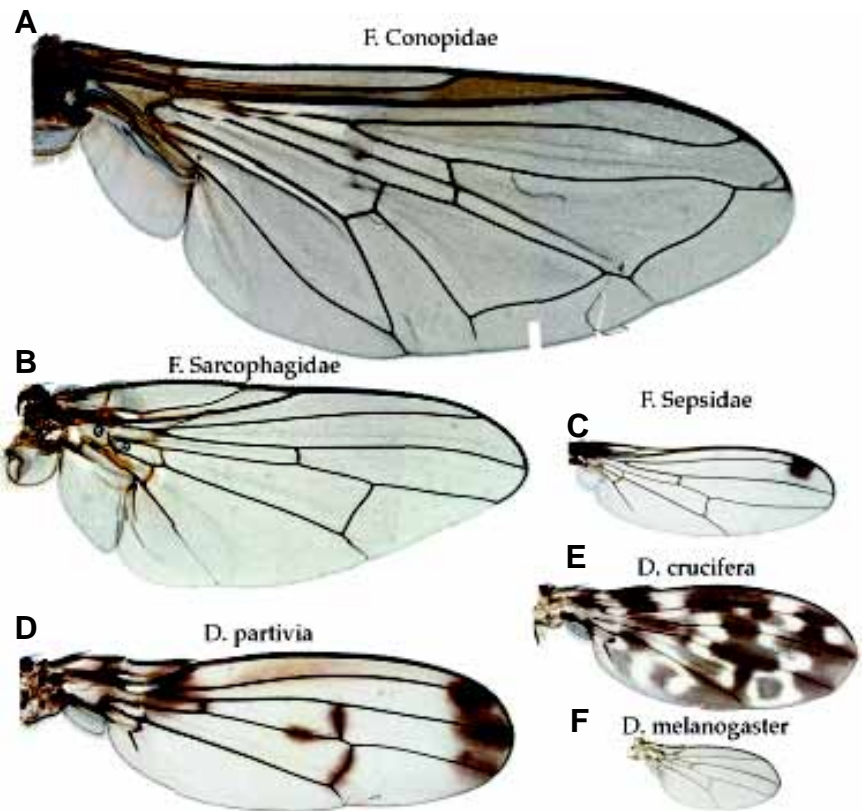


Fig. 5. Relation between venation pattern and wing size. Dipterous flies with similar patterns of longitudinal veins but very different wing sizes belonging to the Families Conopidae (A), Sarcophagidae (B), Sepsidae (C) and Drosophilidae (D-F). All pictures are shown at the same magnification.

absence of R2-5 (see Fig. 3E); Psocoptera where the main branches of the medial and cubital veins are fused; and Diptera where the anal veins are very reduced in number and extent (Fig. 3D). Extreme specialisations by reduction also occurs in Hymenoptera, where some families only have one vein in the position of the Costa in the fore wings, and in many Homoptera, where the Subcosta, Radial and Medial veins are fused in the wing base forming a unique vein (Seguy, 1959; Imms, 1964) (Fig. 4).

5) Formation of closed cells

In most insects the longitudinal veins always reach the wing margin. This generalised pattern is altered in some Diptera and in many Hymenoptera, where some of these veins do not reach the wing margin, and are joined distally by transverse veins forming closed cells (Seguy, 1959; Imms, 1964; Chinery, 1993) (Fig. 4).

6) Vein pattern and wing size

The size of the wing is generally proportionate to the size of the body. Although vein reductions occur very often in small wings, there does not seem to be a stringent correlation between vein pattern and wing size. Thus, when comparing Diptera species belonging to the same family or to different families, wings of very different sizes can have almost identical pattern of veins (Fig. 5). Whether or not there is a connection between the growth of the wing and the patterning of veins, as it seems to be the case for

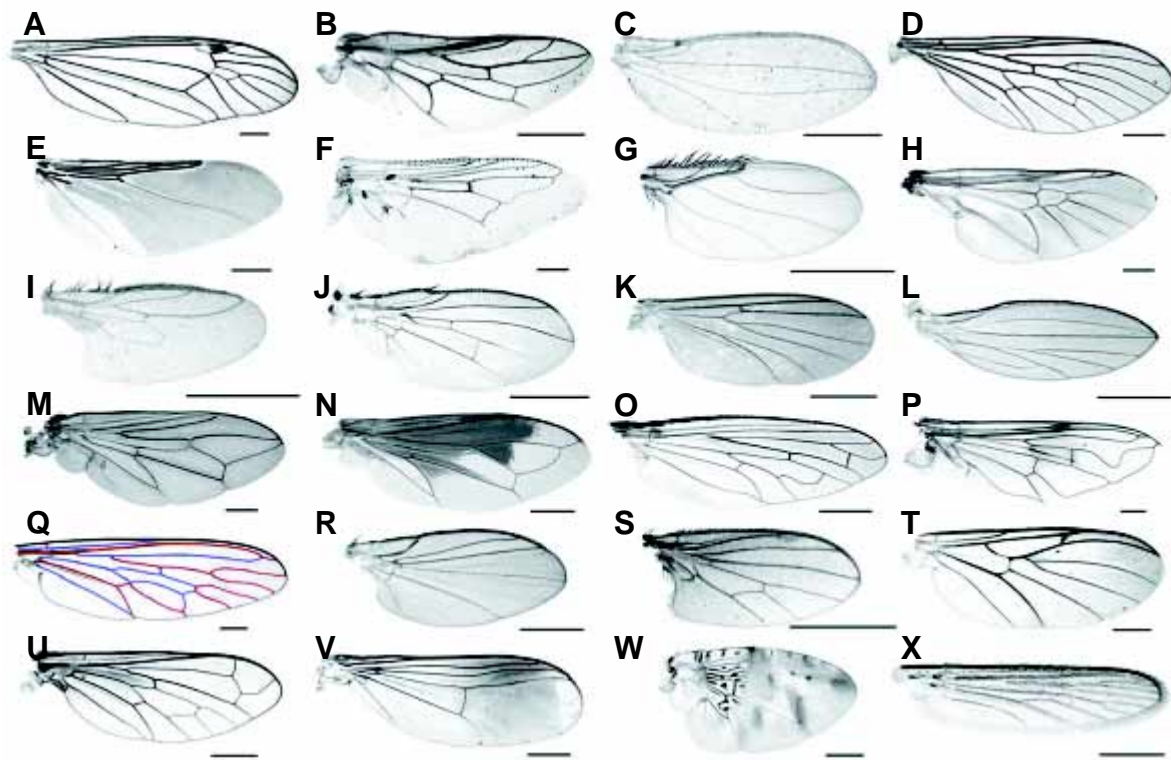


Fig. 6. Vein pattern in Dipterous. Examples of Diptera wings belonging to the Families Tipulidae (A), Scenopinidae (B), Asteiidae (C), Asilidae (D), Hippoboscidae (E), Oestridae (F), Phoridae (G), Stratiomyidae (H), Sphaeroceridae (I), Rhiniphoridae (J), Mycetophilidae (K), Lonchopteridae (L), Conopidae (M,N), Bombyliidae (O), Syrphidae (P), Asilidae (Q), CLusiidae (R), Empididae (S), Mycetophilidae (T), Bombyliidae (U), Dolichopodidae (V), Tephritidae (W) and Culicidae (X).

Drosophila, it has to account for a great flexibility to accommodate the same pattern in different sized wings (Garcia-Bellido *et al.*, 1994; Gonzalez-Gaitan *et al.*, 1994).

Other aspects contributing to variations of wing morphology and appearance related to the veins include patterns of trichome differentiation, the orientation of trichomes with reference to the veins, the formation of pigmentation patterns, the appearance of sensory organs in the veins, and the differences between the dorsal and ventral components of each vein in wings where corrugation is not well developed (see below).

Trends in venation patterns in Diptera

The order Diptera includes more than 100.000 different living species grouped in about 108 families belonging to two major sub-orders named Nematocera and Brachycera. The main characteristic that identified Diptera is the presence of modified hind wings in the form of halteres. The pattern of veins of Diptera varies considerably among families and genera, but is very stable among individuals belonging to the same specie (Stark *et al.*, 1999). Some trends in venation pattern modifications already observed when comparing different orders are also typical of Diptera. Thus, there is a marked correlation between smaller wing sizes and reduction in the number of veins, most variations in vein number and pattern affect distal elements, the connections of the veins and the axillary plates in the wing hinge are constant (see Fig. 2A), and in the more derived families the number of veins is reduced. A detailed consideration of the trends in vein pattern change observed in Diptera in

a vein-by-vein approach have been presented elsewhere (Stark *et al.*, 1999). From the perspective of the *Drosophila* pattern of veins, we have selected several examples of extreme pattern variations with the aim of identifying which rules of construction are operative, and what changes to them might be a source of pattern variation during evolution. We do not attempt to establish particular directions in the variations observed, and therefore the phylogenetic relationships between different species are not considered in this discussion.

Drosophila venation pattern is relatively simple when compared with other insects and consists in four main longitudinal veins (LII-LV) and two short transversal crossveins (cv-a and cv-p) (Fig. 3D). In addition, there is a marginal vein running all along the anterior wing margin and two short veins in the anterior-most and posterior-most areas of the proximal wing (LI and LVI). All these veins are characterized for their corrugation (dorsal or ventral), contacts with the sclerites in the wing hinge and the presence of different types of sensory elements. These three features allow in most cases a clear identification of homologous veins in other Diptera, although in many cases the identification is uncertain.

1) Displacements of pattern elements in the proximal-distal axis The points where veins reach the wing margin and the places where adjacent veins connect to each other or bifurcate in the proximal-distal axis are landmarks subject to many modifications. We consider proximalization or distalization any displacement of these landmarks in the corresponding directions. These changes can be global (Fig. 6A), region specific (Fig. 6B), or vein

specific (Figure 6C). At the same time there are cases in which proximal landmarks are distalized and distal landmarks are proximalized in different regions of the same wing (Fig. 6D). Finally, the identification of vein homologies suggests that pieces of veins can be removed from the proximal wing without affecting the remaining more distal part of the same vein. Modification of the vein pattern in the proximo-distal axis suggests the existence of a positional information system operative along this axis. Most likely, this system is related to the establishment of the dorso-ventral organiser characterised in *Drosophila* by the expression of the secreted protein Wingless at the dorso-ventral compartment boundary (Diaz-Benjumea and Cohen, 1995; Blair, 1995; Irvine and Vogt, 1997). The expression of *wingless* in this region allows the regulation of gene expression relative to the dorso-ventral boundary (the future wing margin), and therefore the positioning of proximo-distal pattern elements (Zecca *et al.*, 1996).

2) Displacements of pattern elements in the anterior-posterior axis

The anterior-posterior (A/P) compartment boundary is a key reference for vein pattern formation in *Drosophila* (Blair, 1995; Lawrence and Struhl, 1996) (see below). The A/P compartment boundary is not associated with any adult morphological feature, runs between the veins LIII and LIV, and separates the two main proximal trunks of veins (Fig. 3D). We assume that compartments are present before the appearance of Pterygota, and therefore that each vein has a compartment identity. This implies that wing venation has an intrinsic symmetry with three main trunks of veins in the A compartment (Costa, Subcosta and Radius) and other three in the P one (Media, Cubitus and Anal). The identification of

the A/P compartment boundary in relation with the pattern of veins shows many cases of asymmetrical growth of each compartment (Fig. 6 G,H,V). Assuming a fixed compartment identity for the veins, and with the caveat of the incertitude to identify correctly vein homologies, several modifications could alter the position of a vein with respect to its ancestral compartmental origin. They include fusion of vein stretches from different compartments and the presence of transverse veins connecting longitudinal veins belonging to different compartments. Furthermore, the topography of the secondary veins that usually appear in the distal-most region of the wing suggests that they might be formed by anterior and posterior cells, resulting in veins "crossing" the A/P compartment boundary (Fig. 6X). In *Drosophila* the posterior vein L4 can be differentiated by anterior cells in several experimental situations, further indicating that compartments do not contain a fixed inventory of pattern elements (de Celis *et al.*, 1995).

3) Loss of longitudinal veins

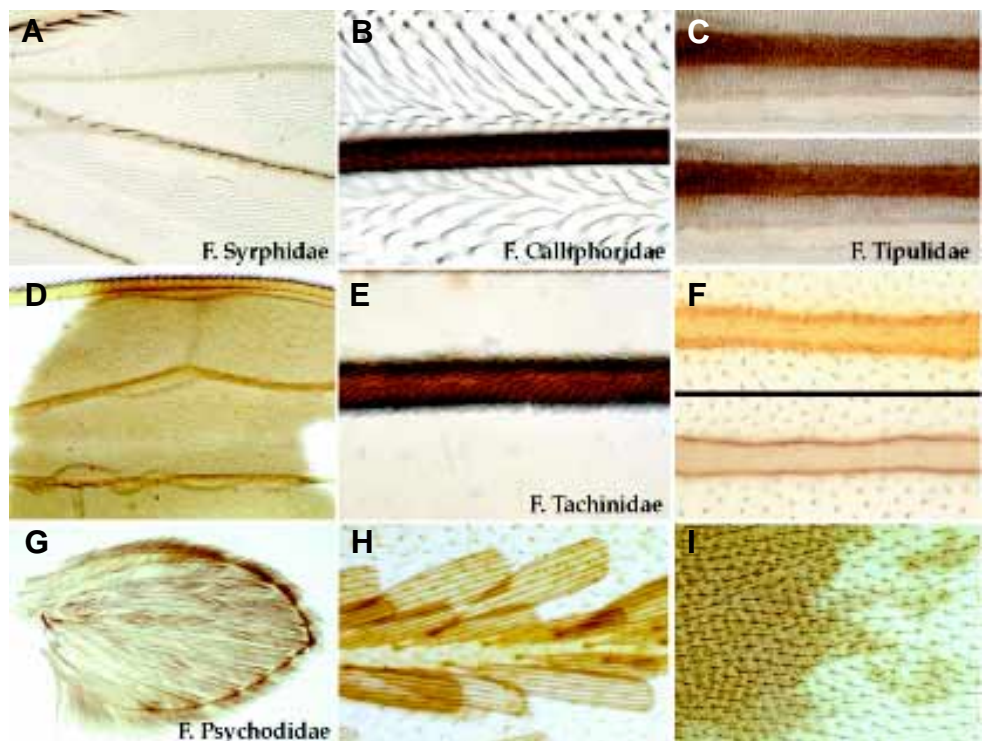
The venation pattern of the Drosophilidae superfamily is one of the simplest one of all Diptera. Nevertheless, there are a few cases of wings differentiating fewer veins, or in which specific veins have been shortened (Fig. 6I). The examination of different patterns of reduction in vein number or length suggests that it takes place regionally. Thus, it is possible to reduce the number of veins either in the proximal-most region (Fig. 6K), in the distal-most one (Fig. 6 I,J), or only in the anterior part of the wing (Fig. 6G).

4) Vein thickening

The size and number of cells forming the vein in width determine the thickness of the veins. Generally vein thickness is

Fig. 7. Variations in cell differentiation associated with the dorsal and ventral components of veins.

(A) Diptera, F. Syrphidae showing bristles in some veins and only in the dorsal wing surface. (B) *O.* Diptera, F. Calliphoridae, illustrating different trichome morphology in the vein and interveins, and trichome orientation in the intervein towards the vein. (C) *O.* Diptera, F. Tipulidae, dorsal (above) and ventral (below) views of the same wing region showing the different differentiation and polarity of trichomes in both surfaces. (D) *O.* Diptera showing irregular thickening of some veins. (E) *O.* Diptera, F. Tachinidae showing extreme variation in cell differentiation between the vein (pigmented and with trichomes) and the interveins (unpigmented and without trichomes). (F) Dorsal and ventral views of the same wing region of a Hymenopterous wing showing extreme differences between vein and intervein trichomes and between dorsal and ventral vein trichomes. (G,H) *O.* Diptera, F. Psychodidae showing differentiation in all the veins of large bristles. (H) is a higher magnification of one vein. (I) *O.* Diptera, F. Conopidae, showing extreme proximo-distal differences in trichome morphology and pigmentation. Distal trichomes look normal (one by cell) whereas in proximal regions each cell differentiates several trichomes.



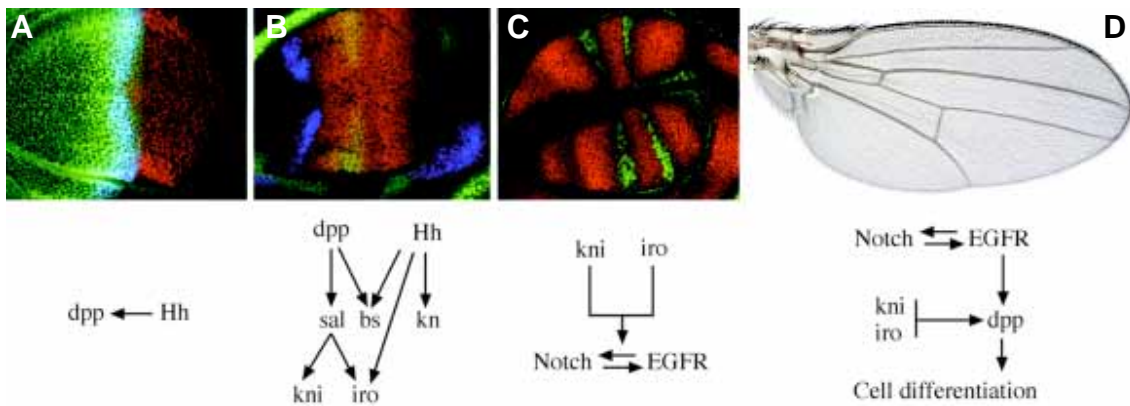


Fig. 8. Main developmental operations characteristic of *Drosophila* vein pattern formation. (A) Activation of *dpp* expression by *Hh* signalling at the anterior-posterior compartment boundary. Expression of the posterior selector gene *engrailed* is in red, the *Hh* target *Cubitus interruptus* (*Ci*) is in green and *decapentaplegic* (*dpp*) expression is in blue. (B) Activation by *Dpp* and *Hh* signalling of *spalt* (*Sal* in red) and *knot* (*kn*; not shown in the picture), respectively, in nested domains in the wing. The function of *Sal* is in part to regulate the expression of the genes encoding the transcription factors *knirps* (*kni*, in blue) and *iroquois* (*iro* in green) in individual veins (*kni* in LII, *iro* in LI, LIII and LV). (C) Expression of *blistered* (*bs*; red) in the interveins and establishment of signalling domains in the veins (green, expression of *argos*, a target of the EGFR pathway). The vein-specific transcription factors are required to activate the expression of several members of the Notch and EGFR signalling pathways. (D) Adult wing and summary of the genetic interactions taking place in the pupal wing leading to the vein-specific expression of *dpp*.

constant in the proximo-distal axis along the wing blade, although there are examples of irregular vein thickening (Fig. 7). Many variations also exist in the number of cells forming the veins in width when comparing different veins in the same wing and the same vein in different species. It is likely that modification in veins thickness results from changes in the efficiency of lateral inhibition between vein cells, as this is the mechanism determining vein width in *Drosophila* (de Celis *et al.*, 1997).

5) Modifications in vein corrugation

Veins differentiate as grooves in either the dorsal or the ventral surfaces, defining the corrugation of the wing. In Diptera, where the wing tends to be flattened, corrugation is referred to the preferential differentiation of vein characteristics in the dorsal or ventral wing surfaces. As a general rule dorsal and ventral veins alternate in the pattern, and this is also observed in the *Drosophila* wing. Nevertheless, when the veins appear in the distal-most part of the wing, they all have the same corrugation (Fig. 6Q). Other modifications to the corrugation include the formation of more symmetrical dorso-ventral veins, and the elimination of vein differentiation in one wing surface (Fig. 7). The corrugation of the extra veins characteristic of several *Drosophila* mutants is always related to the region where these veins appear (Diaz-Benjumea and Garcia-Bellido, 1990). This suggests that the competence to differentiate dorsal or ventral corrugation, which is regulated by the dorsal selector gene *apterous*, is defined by stripes of wing territory and not by single veins (Diaz-Benjumea and Garcia-Bellido, 1990; Milan *et al.*, 2001).

6) The case of the marginal vein

The margin of the wing differentiates a vein that in some cases run all along the wing margin and in other cases ends up in different points. In *Drosophila* the marginal vein runs in the anterior wing margin and finish where the vein LIV reaches the margin. The position of the marginal vein in other Diptera suggests a complex constitution in which this vein is built up from the extension and fusion of different longitudinal veins when they

reach the margin. Thus, in Conopidae, the vein LIV reaches the margin in a more anterior position, and the end point of the marginal vein is also shifted anteriorly (Fig. 6M). In cases where the distal end of LIV is fused with LIII the marginal vein ends up in LIII. In Bombyliidae the marginal vein looks like a continuation of LVI in the posterior compartment (Fig. 6O). Extreme modification of the position of the marginal vein occurs in Syrphidae, where this vein runs parallel and proximally displaced to the wing margin (Fig. 6P). Unfortunately, the corrugation of the marginal vein does not allow answering the question of whether it is a single vein or, alternatively, is formed by the fusion of distal parts of several longitudinal veins.

7) Modification in the crossveins

Crossveins are different to longitudinal veins in that they do not carry tracheae and appear in very constant and characteristic positions compared to other transverse veins (Comstock and Needham, 1999). Furthermore the crossveins are absent in some Diptera (Fig. 6R) and specifically affected in particular *Drosophila* mutants, suggesting that their formation is controlled by different genetic mechanisms than that of longitudinal veins (Diaz-Benjumea and Garcia-Bellido, 1990; Conley *et al.*, 2000). The two crossveins characteristics of the *Drosophila* wing can be found in almost all families of the suborder Brachycera. Most modifications to the crossveins affect the posterior one, where in many Diptera and in some *Drosophila* mutants an extra-longitudinal vein appears from the middle of the posterior crossvein and runs toward the wing margin (Fig. 6S). This pattern suggests that the posterior crossvein is the remnant of two shortened and fused longitudinal veins. In some species of the family Mycetophilidae one longitudinal vein (LII) is shortened and acquires the appearance of a crossvein (Fig. 6T), suggesting that longitudinal veins might be modified into crossveins. We feel, however that the existence in *Drosophila* of a class of genes dedicated to the formation of crossveins implies a clear developmental separation between the crossveins and the longitudinal veins, and allows independent variations in these structures during evolution.

Vein patterning mechanisms in the *Drosophila* wing: developmental operations

From the above summaries, it seems clear that vein patterns are extremely diverse in the details, even if they can be referred to a common ancestor with individual vein branches retaining the same connections with the wing axillary region. It also appears that several trends in venation can diverge independently among orders and families, making impracticable the reconstruction of possible lines of descent between individual patterns. To what extent the study of the mechanisms participating in *Drosophila* vein patterning helps to understand the genetic basis of vein pattern formation in other species, as well as the causes for pattern diversification during evolution, is yet unknown. We expect, however, that vein pattern formation could involve a similar set of developmental operations, even if the known *Drosophila* genes involved in vein formation are not required in the same manner in other insects. In this section, we discuss the mechanisms directing *Drosophila* vein pattern formation, with emphasis in the characteristics that may underlay pattern diversification during evolution. We expect, and it is assumed in the following discussion, that the general operations that characterise wing development in *Drosophila*, such as the existence of compartments with their anterior-posterior and dorso-ventral organisers, are conserved between Pterygota.

Vein patterning in *Drosophila* is intimately related to the development of the wing disc, and involves a succession of stages characterised by the progressive refinement of vein positions (de Celis, 2003). The same cell signalling systems are involved in the growth of the wing imaginal disc and vein patterning (Lawrence and Struhl, 1996). Thus, both the Hedgehog and Decapentaplegic signalling pathways direct the growth of the wing blade and directly contribute to the first step of vein patterning, the positioning of pro-vein territories (Fig. 8 A,B). This early stage is characterised by the regulation of several genes and gene complexes encoding transcription factors in broad longitudinal domains along both the dorsal and ventral components of the future veins (de Celis, 2003). Simultaneously, the future interveins are determined by the localised expression of yet another transcription factor, the Serum response factor homologue Blistered (Fristrom *et al.*, 1994; Montagne *et al.*, 1996; Roch *et al.*, 1998). We would like to point out two characteristics of *Drosophila* vein patterning relevant to vein pattern variations during evolution 1) the specification of each vein occurs by a different transcription factor or combination of transcription factors, and 2) the regulation of *blistered* expression in the interveins is modular (Nussbaumer *et al.*, 2000). The individual identity of each vein, as revealed by the specific genes expressed in their presumptive territories, implies that one particular vein can be affected independently of the others, a trend observed in all orders of insects (Figs. 1,4,6). The existence of region-specific enhancers driving *blistered* expression in individual interveins also implies that the interveins can be modified independently of each other by changes in *blistered* regulation. Thus, the elimination of *blistered* expression in one intervein would result in the fusion of adjacent veins, and the appearance of novel domains of *blistered* expression might underlie vein bifurcations.

Following the subdivision of the wing blade in pro-veins and interveins by the expression of specific genes, a second stage in vein formation is characterised by the deployment of Notch and

EGFR signalling pathways in the borders and centre, respectively, of each pro-vein (Fig. 8C). The activities of these pathways are intimately related, and they contribute to restrict (Notch) and maintain (EGFR) the vein fate (de Celis, 2003). The interplay of Notch and EGFR signalling determines from very early on the thickness of the vein, and it is likely that this or a similar mechanism combining lateral inhibition and activation is ancient and operative to define vein width in all insects. We expect that changes in the parameters and efficiency of such a mechanism could bring about many modifications in the final venation pattern, affecting not only the thickness of the vein but also its differentiation.

The first two operations considered, patterning and signalling within proveins, occur during the growth of the disc, and take place independently and symmetrically in its dorsal and ventral halves (de Celis, 1997). During the growth of the disc, the veins are preferential clonal restriction boundaries, suggesting an intimate relationship between vein specification and growth control (Gonzalez-Gaitan *et al.*, 1994). The third stage in vein formation occurs in the pupal stage, after the eversion of the disc and the apposition of the dorsal and ventral wing surfaces, and it is defined by the restricted expression in the veins of the gene *decapentaplegic* (*dpp*) (Fig. 8D; de Celis, 2003). The critical aspect of this step is the activation of *dpp* expression in each vein, because signalling mediated by Dpp is sufficient and necessary for vein differentiation (de Celis, 1997). Interestingly, the regulatory region driving *dpp* expression at this stage are organised modularly, since individual enhancers drive expression of reporter genes in specific veins (Sotillos and de Celis unpublished). This observation implies that each vein retains, even at this late stage, individual characteristics susceptible to independent variation during evolution.

The last stage of vein formation is less understood, and consists in the differentiation of the characteristics that make vein cells distinct to intervein cells in the adult wing. Thus, vein cells have differential expression of cell adhesion molecules, are more compacted and differentiate a more pigmented cuticle than intervein cells (Fristrom *et al.*, 1993). Cell polarity and particular patterns of cell differentiation are also features that vary among species and within territories of the same wing (see Fig. 7). The variations in final differentiation imply that modulations of several cellular characteristics such as cytoskeleton dynamics, cell adhesion, planar polarity and synthesis of cuticle are also crucial during diversification in evolution.

The analyses carried out in *Drosophila* have led to several hypotheses aiming to explain pattern variations between species. The comparison of *Drosophila* mutant wings with wings in other species suggested that modifications of orthologues to *Drosophila* genes cause pattern resemblances called "phyletic phenocopies" (Stark *et al.*, 1999). For example, in many Diptera the vein L5 (Cu) does not reach the wing margin, a characteristic of several *Drosophila* mutants such as *Hairless* or *abrupt* (Diaz-Benjumea and Garcia-Bellido, 1990). The phyletic phenocopy paradigm suggests that in these Diptera one of these genes is affected in a way that mimics the *Drosophila* mutant phenotype (Stark *et al.*, 1999). We believe, however, that several different genetic changes could theoretically generate the same phenotype. This implies that the predictive power of the phenotype or morphology in regard to its molecular basis is very limited, and indicates that any experimental approach in organisms other than *Drosophila* must include the study of several key genes implicated in each identi-

fied developmental operation. A similar idea to the phyletic phenocopy paradigm is also present in the "paraveins" hypothesis (Bier, 2000). Paraveins are thought to be regions of high vein-formation competence appearing at the boundary of gene expression domains that could correspond to atavisms reminiscent of an ancient pattern (Bier, 2000). It is clear that different regions of the *Drosophila* wing have different competence to differentiate veins in several mutant conditions such as in *extramacrochaetae*, *plexus* and *net* alleles (Thompson, 1974; Diaz-Benjumea and Garcia-Bellido, 1990; de Celis *et al.*, 1995). We have to consider, however, that the wing blade region is extremely heterogeneous in both expression patterns of transcription factors and domains of signalling. Thus, the preferential differentiation of veins in particular places could reflect heterogeneities that are characteristic of the *Drosophila* wing, bearing little or no relation to a primitive vein patterning system.

We feel it is more constructive to think about vein pattern variations in terms of the developmental operations identified in *Drosophila*. These can be summarised as follow: 1) patterning of the disc epithelium consisting in the generation of a landscape of transcriptional regulators. In this stage signalling molecules such as Dpp and Hedgehog, the transcription factors regulated by them, and the regulatory interactions between these targets define cellular territories with different fates (individual proveins and interveins). 2) Establishment of interdependent domains of signalling leading to the partition of the provein into adjacent domains. 3) Activation of *dpp* expression in the veins, using different enhancers for each vein. 4) Regulation of cell adhesion and shape in vein and intervein cells, and differential expression of vein characteristics in the dorsal and ventral components of each vein. These steps are related hierarchically, in the sense that the result of each operation determines and conditions the deployment of the successive one. They are also related combinatorially, because two or more parallel inputs contribute to convey information about dorso-ventrality, pigmentation and polarity to vein and intervein cells. The extreme variation in detail shown by venation patterns and the recurrence of similar modifications in distant species must be related to the number of genes involved in each step, as modifications in those genes or in the regulatory circuitries linking them are susceptible to be the cause of pattern variations. We believe that pattern diversification is based on the individual specification of each vein, the existence of common mechanisms affecting all veins and the modular structure of the regulatory regions of the key genes. These three characteristics offer many candidate entry points for modifications affecting the vein pattern globally, as well as modifications affecting individual veins or interveins independently. Assuming a general conservation of the processes involved in different species, the transitions between very different patterns may only require few changes in the regulatory gene networks involved.

Acknowledgements

We would like to thank Rosario Hernandez and the Department of Zoology of the Universidad Autónoma for the preparation of the specimens shown in Figures 3, 4, 5 and 7. Also, we thank Isabel Izquierdo and Carolina Martín, managers of the collection of entomology of the Natural History Museum (Madrid), which kindly provided to F. D-B all the facilities for the analysis of Diptera specimens shown in Figure 6. We also thank A. Garcia-Bellido and all members of his laboratory for critical comments on the manuscript.

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