

## Butterfly wings: Colour patterns and now gene expression patterns

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### Summary

The particular fascination of butterfly wings for developmental biologists (and others) lies in their spectacular array of colour patterns. The evolutionary and developmental relationships between these patterns have been analysed and we know something of the cell interactions involved in their formation<sup>(1)</sup>. Now butterfly homologues of *Drosophila* wing-patterning genes have been identified, and their expression patterns offer the first clues to the molecular mechanisms which specify wing colour patterns<sup>(2)</sup>.

### Background

Spatial organisation is fundamental to development, but what are the mechanisms that ensure that cell differentiation depends upon position? This general question is dramatically posed by butterfly wings, with their striking colour patterns. Wing development in butterflies probably starts, as in *Drosophila*<sup>(3)</sup>, from a small group of cells in the early embryo. Within the larva, these cells form a growing pouch, or 'imaginal disc', consisting of the two layers of epidermis which will form the dorsal and ventral surfaces of the wing (the arrangement is rather different in *Drosophila*). At metamorphosis, the pouch protrudes to form the simple pupal wing and, late in the pupal stage, the epidermal cells form elongated, pigmented scales that will cover the adult wing. The wing colour pattern therefore presents a vivid record of spatial differences within the pupal wing epidermis.

Butterfly colour patterns are very diverse and mostly extremely complicated; not surprisingly, experimental analysis has concentrated on such simple components as the eyespot (Fig. 1). By surgically manipulating the epidermis of *Precis coenia*, Fred Nijhout showed that pattern forms independently on the two wing surfaces and, furthermore, that the complete posterior eyespot pattern is specified at early pupal stage by signals coming from a 'focus' at its centre<sup>(4)</sup>. Thus, cauterising that focus could remove the adult eyespot and, critically, grafting it elsewhere induced an ectopic eyespot pattern. The focus itself must have been established earlier, during larval development of the imaginal disc. Other elements of wing pattern, such as the bands (Fig. 1), seem to have been completely specified in the imaginal disc, as they were not influenced by pupal operations<sup>(4)</sup>.

Nijhout suggested that the focus signals by producing a

molecule (traditionally but unhelpfully termed a 'morphogen'), which diffuses away to form a concentration gradient in the surrounding pupal epidermis. If later scale colour depends on a cell's morphogen level, the gradient contours will define rings of an eyespot pattern<sup>(4,1)</sup>. Patterning could occur also by short-range interactions, but our results from another butterfly, *Bicyclus anynana*, argue against this mechanism. Thus, in grafts of the central foci of large and small eyespots, the size of an induced eyespot depends mainly on the identity of the focus, not on the responding epidermis<sup>(5)</sup>. This suggests that the eyespot focus provides one long-range signal, rather than merely the first of a cascade of short-range signals.

The eyespot is very simple in relation to the kaleidoscope of butterfly wing patterns. Building on work of the 1920s, however, Nijhout argues persuasively for simple conserved mechanisms, for 'order beneath the exuberant diversity'<sup>(1)</sup>. He has analysed wing patterns as variations of a basic 'groundplan' that has pattern elements repeated in each 'wing-cell' (between successive wing veins). Much of the diversity lies in the absence of elements (Fig. 1) and in their shape, which may depend on slight differences in signalling from foci in a few standard positions. The eyespot would result from the simplest situation of a conical gradient from one midline focus. The focal positions could be established in each wing-cell by a diffusion-reaction mechanism, in relation to the bordering veins and wing margin<sup>(1)</sup>.

### Gene expression patterns and colour patterns

In recent years, *Drosophila* molecular genetics has been the major route to an understanding of developmental mechanisms. Mutant phenotypes implicate particular genes in, for example, the development of imaginal discs. Subsequent study can reveal when and where these genes are expressed, how they are regulated and their probable function. Thus, we now know a lot about genes important in the development of the (drably monochrome) *Drosophila* wing<sup>(3,6)</sup>. Recently, Sean Carroll and colleagues have isolated homologues of some of these genes from *Precis* and have started to relate gene expression patterns to the specification of the butterfly colour patterns<sup>(2)</sup>.

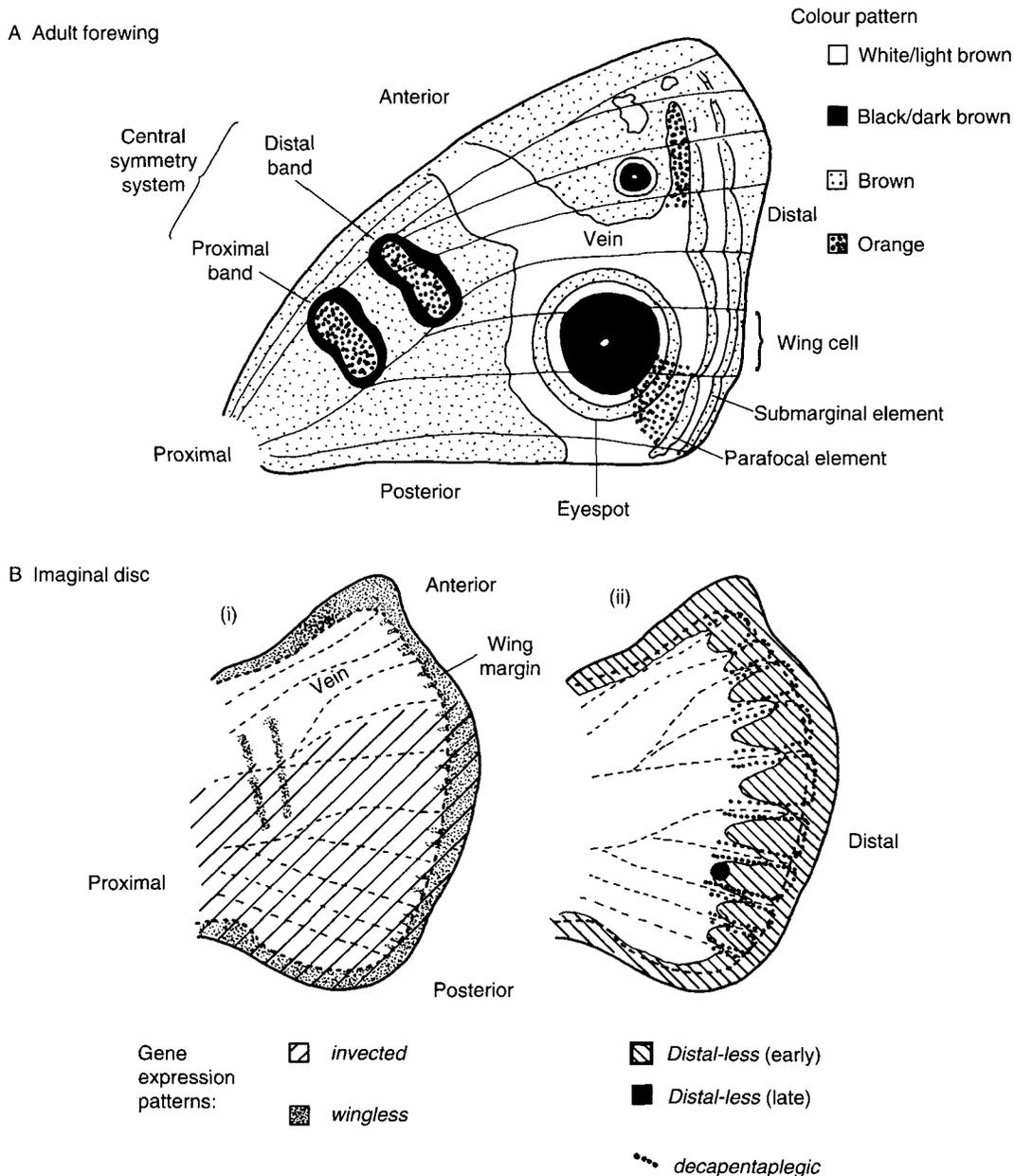
Many features of gene expression in the wing imaginal disc are conserved between *Drosophila* and *Precis*<sup>(2)</sup>. The genes *apterous* and *engrailed* are transcribed, respectively, within dorsal and posterior halves (compartments) of the *Drosophila* disc, and the same is true (strictly, for homologues of *apterous* and the *engrailed*-related gene, *invected*) in *Precis* forewing and hindwing discs. The *Drosophila* gene *wingless*, is expressed in the mature disc as a narrow stripe around the future distal wing margin (and in regions of the proximal wing blade and hinge). In *Precis*, *wingless* is also expressed around the distal margin of the disc, mostly in peripheral epidermis which will not contribute to the adult wing. There may be a difference, however, involving the gene *decapentaplegic*, which is expressed in a stripe along the edge of the anterior compartment in the *Drosophila*, but apparently not in the *Precis*, wing disc<sup>(2)</sup>.

Other aspects of gene expression in the *Precis* wing disc

## WHAT THE PAPERS SAY

have no parallel in *Drosophila* and may relate to features of the colour pattern that is formed much later. The most striking correspondence concerns *Distal-less* and the eyespot focus (Fig. 1). Midway through the last larval stage, *Distal-less* is expressed distally (as it is in the *Drosophila* mature disc<sup>(3)</sup>), but as broad rays extending from the margin down the midline of each wing-cell. Most of these rays then fade away, but transcription persists, on all wing surfaces, in a spot at the future centre of the posterior eyespot<sup>(2)</sup>. Sur-

prisingly, *Distal-less* expression does not persist at the centres of the anterior eyespots, which are also present on all adult wing surfaces. There are two proximal stripes of *wingless* expression on the forewing disc, in positions which may correspond to the adult wing bands<sup>(2)</sup>. Also, in each wing-cell, there are features which do not correspond to the later colour pattern: expression of *wingless* in two short distal rays and, probably, of *decapentaplegic* in longer rays (Fig. 1).



**Fig. 1.** Adult colour pattern (A) and larval gene expression patterns (B) on the dorsal surface of the forewing of *Precis coenia*. The *Precis* colour pattern displays only some of the components of the 'groundplan' (see text), in which there are submarginal, parafocal and eyespot elements (of diverse shapes) in each wing-cell, and the bands of a central and a proximal symmetry system stretch completely across the wing<sup>(1)</sup>. The representations of gene expression (data derived from *in situ* hybridisation with probes to gene transcripts<sup>(2)</sup>) show separately the patterns for (i) *wingless* and *invected* and (ii) *Distal-less* and *decapentaplegic*.

## Beyond expression

From the gene sequence, the general function of the protein can often be deduced and, in *Drosophila*, the use of mutations and manipulations of gene expression can suggest the developmental role. Thus, *apterous* and *engrailed* contain homeobox motifs and therefore probably control directly the expression of other genes. In *Drosophila*, their expression specifies regional cell fate (i.e. 'dorsal', 'posterior') and initiates cell interactions across the dorsal/ventral and anterior/posterior compartment borders<sup>(3,6,7)</sup>. *wingless* and *decapentaplegic* encode secreted proteins that act as signals in these interactions, regulating both cell division in the imaginal disc and the pattern (of veins, bristles, etc.) of the future wing<sup>(3,6,8)</sup>. Homology of gene structure and similarity in expression argue enticingly for homology of function, and these genes may well perform similar roles in wing development in *Drosophila* and *Precis*<sup>(2)</sup>. What, then, of the colour patterns?

The proteins encoded by *wingless* and *decapentaplegic* probably function as intercellular signals, here as in *Drosophila*. Carroll *et al.*<sup>(2)</sup> suggest that their localised production, in each wing cell and in a pattern related to the wing veins, could be part of the mechanism, conserved among butterflies, of establishing focal positions for the subsequent specification of colour pattern<sup>(1)</sup>. To investigate this possibility, it will be important to study gene expression throughout disc and pupal wing development (not just in the last larval stage), in *Precis* but also in species with different adult colour patterns.

In the *Precis* late wing disc, *Distal-less* is expressed in the posterior 'focus'<sup>(2)</sup>, the region which subsequently specifies the posterior eyespot<sup>(4)</sup>; what might be its role in eyespot formation? The protein is a putative transcription factor (certainly not a diffusible morphogen!) but there are indications that it could function indirectly in cell interaction. *Distal-less* is expressed in the embryonic and larval leg discs of *Drosophila*<sup>(3)</sup>, in a central region corresponding to the future distal part of the leg. *Distal-less* mutants lack distal leg structures, and the phenotype of mosaic animals (with only a clone of mutant tissue) indicates that *Distal-less* function is required for short-range cell interactions that pattern the distal part of the leg<sup>(3,9)</sup>. The precise role of *Distal-less* remains unclear and, curiously, no role is indicated for its expression in the *Drosophila* wing!

Carroll *et al.* argue from the *Distal-less* expression patterns (it is also expressed in *Precis* embryonic legs<sup>(10)</sup>) that the distal-proximal axis of the leg and the radial axis of the wing eyespot may develop by similar mechanisms<sup>(2)</sup>. Perhaps!

The *Precis* gene expression patterns appear to give intriguing

glimpses of pattern formation in butterfly wings. The general similarities with *Drosophila* suggest conservation of the basic mechanisms of wing development between the two types of insect (perhaps not surprising!). Other features of expression invite speculation about how familiar genes may be employed in the additional mechanisms which decorate the wings so lavishly. Expression data, however, is a first step which, alone, can only give circumstantial evidence (and there are several cautionary tales from *Drosophila* where particular aspects of the expression pattern seem to be irrelevant to normal function). In *Drosophila*, the role of particular genes, and the relationships between them, can be teased out by removing gene function and manipulating, in various ways, gene expression. In order to get firm evidence for mechanisms of wing colour patterning, we shall need to develop these methods in butterflies.

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