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# Rethinking Butterfly Eyespots

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**Abstract** Evo-devo seeks to explain the origins of novelties in terms of genetics. Butterfly eyespots offer a fertile subfield for such investigations. Previous explanations for the origin of eyespots are reviewed, and a new hypothesis is presented. According to this new "Recursion Model," eyespots are ectopic versions of the wing margin. Evidence for this equivalence includes: (1) secretion of the morphogen Wingless, (2) expression of the homeobox gene *Distal-less*, and (3) specification of outlying contours that take the form of stripes or rings. These three steps constitute a modular program that was initially executed only at the margin. The model proposes that eyespots were created when the program was accidentally rebooted (recursively) at certain points in the wing blade by a fortuitous mutation that occurred at the dawn of the Nymphalid family. Those points are located wherever two interacting genes are expressed. Gene *A* is expressed midway between adjacent wing veins, while gene *B* is expressed at a certain distance from the wing margin. The mutation is thought to have installed a new *cis*-enhancer at the *wingless* gene locus, which was uniquely responsive to the combination of *A* and *B* inputs. Because the postulated enhancer should be easy to pinpoint by transgenic in vivo assays of reporter constructs, this new model is directly testable. If it proves correct, then eyespots would become one of only a few putative cases where a novel feature arose suddenly.

**Keywords** Evo-devo · Butterfly · Eyespots · Novelty · Co-option · Heterotopy

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Eyespots are markings that mimic vertebrate eyes (Stevens 2005). In some butterflies they scare off birds (Vallin et al. 2007). In others they deflect an attack away from the head (Olofsson et al. 2010), allowing the butterfly to escape, albeit with a tattered wing (Vallin et al. 2011). However, most eyespots are too small and numerous to play any anti-predator role whatsoever. They might be assisting in species recognition or mate selection (Oliver and Monteiro 2011), or they may just be adaptively neutral spandrels (Gould and Lewontin 1979). That is, they may be accidental byproducts of development. Are eyespots bona fide novelties? This question cannot be answered by definitional criteria (Hallgrímsson et al. 2012) until we know more about the history of their utility (their "evo-" side) and the mechanism of their construction (their "devo-" side). On both fronts we have much to learn.

How did eyespots originate? The genome is the best place to look for clues, but which genes should we investigate?

## How did the Butterfly Get its Spots?

In 1994 the gene *Distal-less* (*Dll*) was shown to be expressed at the center of eyespots in the butterfly *Precis coenia* (Carroll et al. 1994), and later work confirmed this result in other species (Monteiro et al. 2006; Shirai et al. 2012). This finding was startling because *Dll* is best known for causing the outgrowth of appendages throughout the insect world (Panganiban et al. 1994) and beyond (Panganiban and Rubenstein 2002). Might there be a link between eyespots and legs? Sean Carroll and his coworkers who made this discovery clearly thought so (Carroll et al. 1994):

> Indeed, the eyespot may be a proximodistal element superimposed on the two-dimensional wing surface.

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That is, the center of the eyespot (the focus) may represent the distal-most positional value (and express Dll), and the surrounding rings may represent progressively more proximal positions in a manner analogous to the organization of the *Drosophila* leg imaginal disc.

Their proposal will here be called the Flat Leg Model (Fig. 1a, b; Saenko et al. 2011). It connotes both co-option and heterotopy. Co-option is the recruitment of an old circuit (leg program) for a new function (wing patterning; True and Carroll 2002), while heterotopy is the redeployment of an old circuit (leg program) at a new place (wing blade; Arthur 2011). One obvious difficulty comes to mind: there are no butterflies with bumpy wings, so what prevents *Dll* from causing eyespots to grow into leg like appendages (Gorfinkiel et al. 1997)? Perhaps *Dll* turns ON too late to incite mitoses in the wing epidermis?

An alternative scenario is outlined below that also entails co-option and heterotopy, but instead of the leg program, it invokes the gene circuits that normally operate at the wing margin. This proposal is named the Recursion Model (Fig. 1c, d) because it entails a reiteration of the same patterning steps that were executed previously (at a different site) in the same developing organ (wing imaginal disc). Two other hypotheses will also be considered. Before delving into the particulars, however, it might be well to offer a primer on insect development and genetics.

Flies and butterflies belong to different orders of insects—Diptera and Lepidoptera, respectively. Both groups exhibit a holometabolous type of development where they begin life as a feeding larva (maggot or caterpillar) and then transform into a winged adult (fly or butterfly) via a quiescent pupal stage (case or cocoon). The wings grow inside the larva as sacs called imaginal discs (Held 2002). During metamorphosis, wing discs evert, expand, and flatten. Leg discs have concentric folds instead that telescope out to form a cylinder.

In *Drosophila*, genes are named for their mutant phenotypes. The gene *wingless* (*wg*) is a case in point. Disabling *wg* aborts wing development. Wingless is secreted by cells along the wing margin and diffuses proximally along what will be the upper (D, dorsal) and lower (V, ventral) surfaces of the wing blade. (Genes are italicized, but proteins are not, and proteins are capitalized, but genes can be upper or lower case, depending on the nature of their mutations.)

Wingless is termed a morphogen because it *gen*erates differences in *morpho*logy (or pattern) via its concentration (Tabata and Takei 2004). Cells near the margin detect a higher level of Wg than those near the base because they are closer to the source, and cells in between can assess their positions by measuring the amount of Wg at their location. Both flies and butterflies use Wg in this way to specify cell positions along the D–V axis (Carroll et al. 1994). Flies use a separate morphogen for the orthogonal (anterior–posterior, A–P) axis. The A–P morphogen in butterflies is not yet known.

Insect wings are reinforced with rigid veins that branch from the base and extend to the tip in roughly parallel lines (de Celis and Diaz-Benjumea 2003). Butterfly wings are covered with scales, each of which has a single color, whereas fly wings are smooth and transparent (Ghiradella 2010). The panoply of designs we see on butterfly wings is due to scale cells interpreting their A–P and D–V coordinates in different ways from one species to the next (Carroll 1997). How did evolution repeatedly reprogram the pixels to "paint" so many different patterns? No one knows (Brakefield 2007).

Surprisingly, many of these patterns can be reduced to only a few types of variations on a common theme (Schwanwitsch 1924). A "groundplan" was distilled in 1924 by B. N. Schwanwitsch for Nymphalids and related families (Fig. 1j) and was later confirmed in all of its key features by F. Süffert (Schwanwitsch 1929). In the plan there is a single, transverse chain of eyespots, plus multiple parallel stripes. The middle stripes exhibit mirror symmetry (M, G, D, D, G, M), so they are said to comprise a "central symmetry system" or central ribbon.

One added variation is a dislocation of parts of the central ribbon where it crosses a vein in some species, as if the veins were chopping the ribbon into pieces that then slide freely relative to one another along the proximal–distal axis (Nijhout 1978). Fred Nijhout, who has written the classic tome on butterfly wing patterning (Nijhout 1991), thinks that the eyespot chain could have arisen by a similar fragmentation of a distal ribbon that prefigured the chain (Nijhout 2001). His proposal will here be termed the Symmetry Model (Fig. 1e, f).

The final hypothesis to be considered was devised by Antónia Monteiro, another patterning pioneer (Monteiro 2008). She argues that the eyespot chain was assembled one spot at a time, rather than all at once. Different spots are supposed to be controlled by separate *cis*-enhancers at a hypothetical *eyespot master control gene* in the same way that different stripes of *even-skipped* (*eve*) expression are regulated in the *Drosophila* blastoderm by modular enhancer elements near *eve* (Wilczynskia and Furlong 2010). Her scenario will be called the Modularity Model (Fig. 1g, h).

## Evaluating the Extant Explanations

The bull's eye of an eyespot is called its "focus." The focus is necessary and sufficient for induction of the

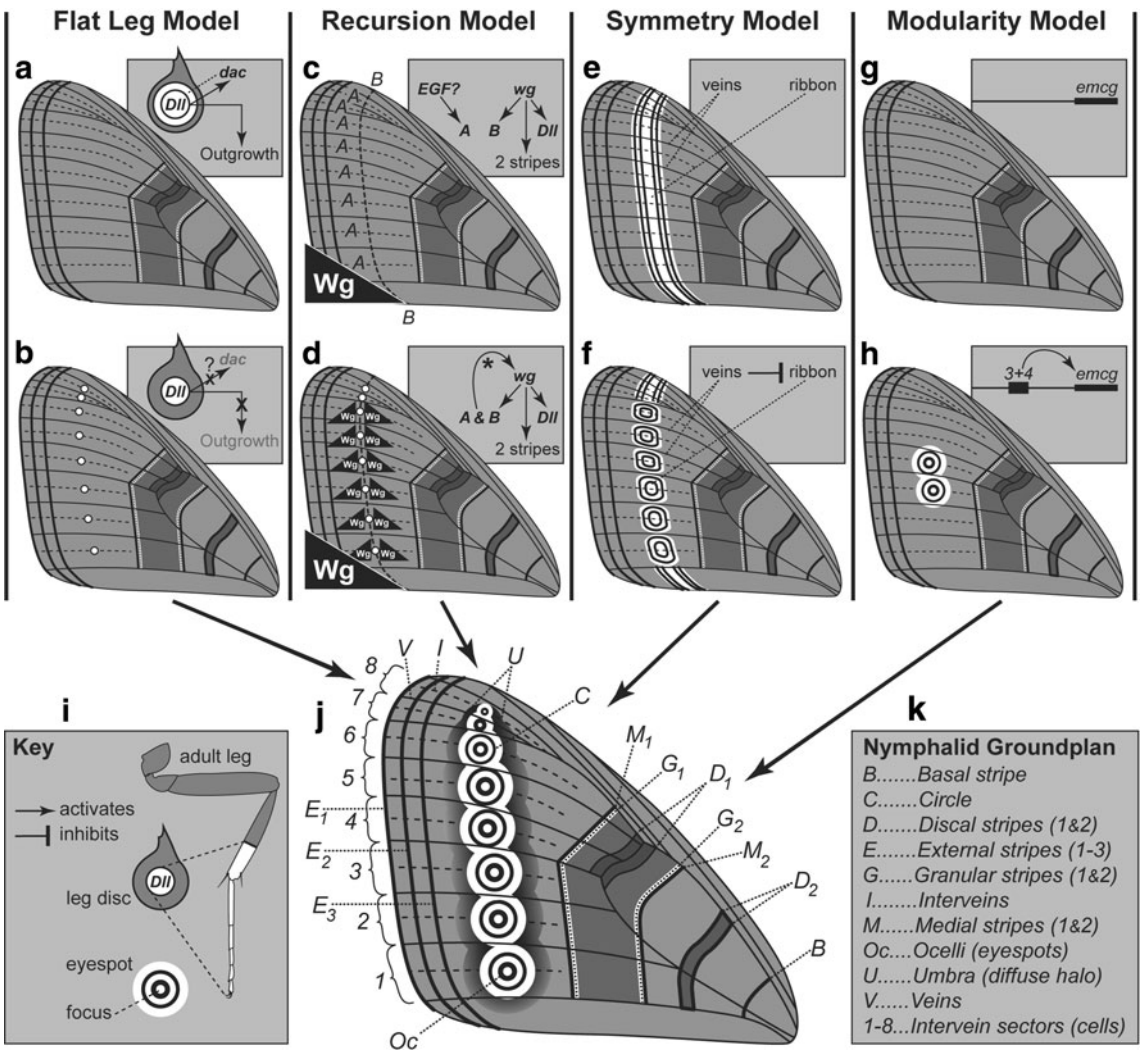


**Fig. 1** Alternative models for the origin of eyespots in Nymphalid butterflies, using Schwanwitsch's groundplan for dorsal forewings (**j**) as the final product. **a**, **b** Flat Leg Model (Carroll et al. 1994). The teardrop-shaped organ in the *gray box* is a fruit fly leg disc, where *Distal-less* (*Dll*) is expressed in the center and *dachshund* (*dac*) is expressed around it (**a**; Giorgianni and Mann 2011). This area forms the leg tip (**i**). Foci (*white dots*) arose (**b**) when a mutation caused *Dll* (but not *dac*?) to be expressed at intervein sites in such a way that outgrowth was blocked (X). **c**, **d** Recursion Model (*cf.* Fig. 5). A gradient (*triangle*) of the morphogen Wingless (Wg) extends proximally from the margin. It actually spans the whole wing (Fig. 2d). Different Wg concentrations do several things (**c**): (1) turn ON *Dll* (Fig. 2a), (2) specify two border stripes ($E_2$ and $E_3$ in **j**), and (3) activate the hypothetical gene *B* (*dashed line*). *Gray box* shows circuitry, including activation of gene *A*, which is expressed midway between adjacent wing veins. Foci (*white dots*) arose (**d**) when a mutation (*asterisk*) caused cells expressing both *A* and *B* (intersection points) to turn ON *wg* (recursion) and hence to make 2 "stripes" that became the concentric rings of the eyespots. **e**, **f** Symmetry Model (Nijhout 2001). The Nymphalid progenitor (**e**) is presumed to have had a distal ribbon like the central ribbon (MGDGM in **j**). A mutation caused the veins to inhibit the ribbon (*gray box*) so that its fragments became eyespots (**f**). **g**, **h** Modularity Model (Monteiro 2008). Initially (**g**) the wing had no eyespots because the *eyespot master control gene* (*emcg*) was constitutively turned OFF. Mutations led to *cis*-enhancers nearby (**h**) that turned *emcg* ON at certain sites (*e.g.*, spots 3 and 4). **i** Key. *Dll* is turned ON in the center (*white circle*) of the leg disc (teardrop), which telescopes out to form the distal half of the leg during metamorphosis (Campbell and Tomlinson 1998). *Dll* is also ON in the center (focus) of the eyespot. **j**, **k** Groundplan for the dorsal forewing of Nymphalids (Schwanwitsch 1924). Redrawn from Schwanwitsch (1929). Note the similarity of eyespot rings to margin stripes ($E_2$ and $E_3$), which inspired the Recursion Hypothesis (**c**, **d**). Numbering of spots (rear to front) is opposite to modern convention

eyespot as a whole. The evidence for this conclusion is that (1) cauterizing a focus can abort the entire spot (French and Brakefield 1992), and (2) transplanting a focus can induce an ectopic eyespot in the surrounding host tissue (Nijhout 1980; French and Brakefield 1995), provided that the area is competent to respond (Beldade et al. 2008).

In 1978 Nijhout proposed that each focus secretes a morphogen whose concentration decreases with distance (Nijhout 1978). Concentric rings of pigment would emerge if the morphogen activates pigment genes at specific levels, like the contour lines surrounding a hilltop in a topographic map.

Here we encounter the first major challenge to one of the four models, and ironically, it is Nijhout's own scenario that is put on the spot, so to speak. His Symmetry Model contends that eyespots came from a ribbon that broke into pieces (Fig. 1e, f). If so, then how did each piece acquire a focus?

The easiest way out of this dilemma would be to assume that veins not only slice the ribbon wherever they cross it but that they also emit a diffusible inhibitor that modifies the inside of each piece as well. The ribbon would be like a ridge that gets chiseled into a mountain chain, with the intervening valleys being carved by the veins (Nijhout 2001).

For this explanation to be valid, all ribbons (including the central ribbon) should secrete the same morphogen from their symmetry plane (to set up their parallel stripes) as eyespots secrete at their foci (to create their concentric rings). Do they?

Until 2006 the identity of the morphogen used by eyespot foci was unknown. In that year Monteiro and her collaborators demonstrated the presence of Wg in the foci of *Bicyclus anynana* (Fig. 2). Earlier researchers had failed to detect Wg at these sites because they had not looked during the brief (6-h) period (early pupal stage) when Wg is produced. Monteiro et al. also showed that the foci must be responding to a TGF-$\beta$ signal of some sort, but that signal is not emanating from the focus per se. The conclusion that Wg is the focal morphogen is only tentative, of course, since other candidates exist that remain untested, but it agrees with Wg's documented roles in (1) modulating eyespot size (Saenko et al. 2010) and (2) inducing pigment spots at vein sites on fly wings (Werner et al. 2010).

Is there a Wg stripe in the central ribbon, as the Symmetry Model implies? Monteiro et al. did not see one in *B. anynana*, but another team later found one in several other Nymphalids (Martin and Reed 2010). As comforting

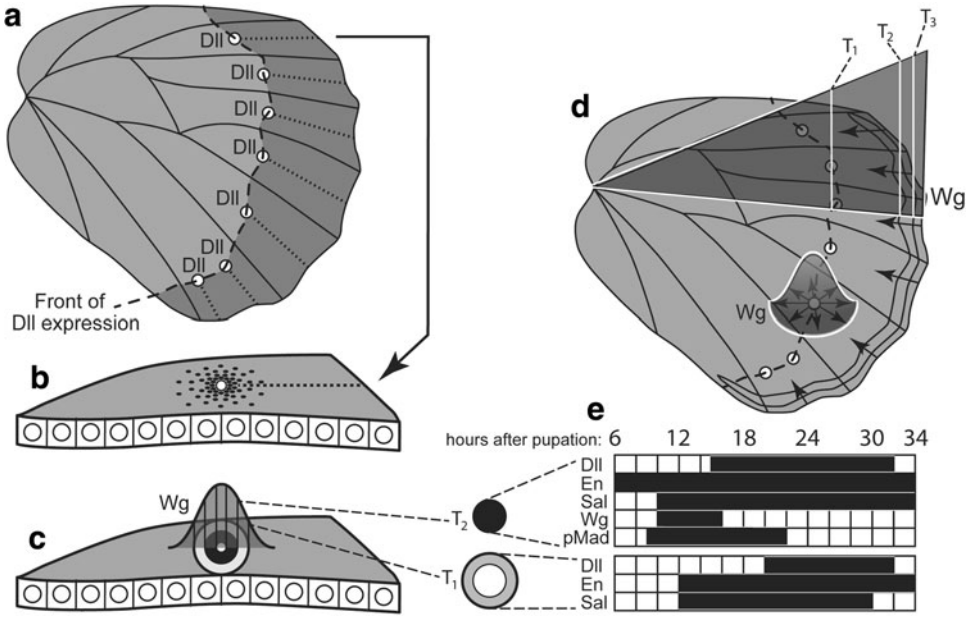


**Fig. 2** Development of eyespots on the ventral surface of the *Bicyclus anynana* left hindwing. Branching lines are wing veins. **a** Dll is diffusely expressed in a zone (up to *dashed line*) bordering the wing margin, as well as being intensely expressed midway between adjacent veins and in a spot that becomes the focus (Brakefield et al. 1996; Nijhout 1996). **b** Enlarged view of one intervein sector, showing the Dll "lollipop" (*dashed line* and *circle*). Hypothetical morphogen molecules (*tiny ovals*) diffuse radially away from the focus. *Squares* with *inner circles* represent epidermal cells (size exaggerated) that form a monolayer. **c** Presumptive hill-shaped gradient (parabola in cross section) of the morphogen Wingless (Wg) (Monteiro et al. 2006). Light or dark pigments are induced at low ($T_1$) or high ($T_2$) thresholds (*vertical lines*). **d** Two types of Wg gradients on the *B. anynana* wing: (1) a basipetal one (*triangle*) spanning the wing and (2) a hill-shaped one at each focus. Only one focal gradient is drawn. *Arrows* indicate directions of diffusion. Both types of gradient elicit specific cell responses at different levels. The triangular gradient, which is actually 3-dimensional (its ridge runs runs along the wing edge and it tapers to the wing base), elicits pigment at $T_2$ and $T_3$ and turns ON gene *B* (*dashed line*; Figs. 1c and 5a) at $T_1$ (edge of the Dll front; **a**). The hill-shaped gradient creates concentric rings (**c**) or turns ON certain genes (**e**) at thresholds $T_1$ and $T_2$. *N.B.*: Wg comes from a linear source for the larger gradient, but a point source for the smaller ones. This reusage of Wg in the smaller gradients led to the Recursion Model (Fig. 5). The two types of gradients would have to act at different times to avoid interfering with one another. **e** Timecourse of protein expression in the focus (*upper panel*) or outer ring (*lower panel*). *Black bars* are time spans when proteins were detected, though ON and OFF times are not so sharp, nor is expression so uniform. Abbreviations: *Dll* Distal-less, *En* Engrailed, *Sal* Spalt, *Wg* Wingless, *pMad* phosphorylated Mad. Redrawn from Monteiro et al. (2006), whose paper should be consulted for details

for the model as this Wg stripe may seem, it only coincides with the $D_1$ zone, and $D_1$ only spans roughly a third the length of the central ribbon (Fig. 1j). Thus, the expectation of a morphogen "ridge" extending along the whole ribbon is not met. In these same species, a second Wg stripe was found at $D_2$, but the significance of that stripe is unclear since $D_2$ is not flanked by satellite stripes like $D_1$.

Assuming that Wg is the focal morphogen, *wg* could be the master control gene of Monteiro's scheme. If so, then the Modularity Model could be tested by looking near the *wg* gene for *cis*-regulatory enhancers that target Wg expression to particular eyespot locations (Conceição et al. 2011). Unfortunately, we don't know what motifs to look for since no "area codes" have yet been deciphered for butterfly wings. A similar ignorance prevents us from probing *Wnt* (*wg*) loci in humans for enhancers that target hair to our scalp, armpits, groin, etc. (Held 2010).

Historically, enhancers have been identified more often indirectly by genetics than directly by genomics (Aerts 2012; Hardison and Taylor 2012). The power of the loss-of-function approach is best illustrated by the Achaete–Scute Complex, which was meticulously dissected by the mapping of bristle-loss mutations (Gómez-Skarmeta et al. 1995). A preliminary study of this kind was conducted by Monteiro's group (Monteiro et al. 2003). They X-rayed wild-type *B. anynana* males and examined their offspring for defective patterns. Four types of spot-loss phenotypes were recovered for the ventral hindwing (eyespots numbered 1–7 from anterior to posterior): 3 and 4 missing, 1, 3 and 4 missing, 1, 2, 3, and 4 missing, and all 7 missing (Fig. 3).

None of these mutations has yet been mapped. Conceivably, each of them knocks out a subset of enhancers at the master control locus (*wg*?). If so, then the fact that spots 3 and 4 are always either both present or both absent suggests that these spots share a single "3 + 4" enhancer (Fig. 1h).

The Modularity Model assumes that (1) the ancestor of all spot-bearing species lacked eyespots, and (2) its descendants added spots in discrete steps by acquiring spot-specific enhancers until they achieved the full quota we see today. The fact that these mutants can be seriated by spot gain (or loss) is consistent with both tenets, but a recent phylogeny of Nymphalids is not (Fig. 4; Kodandaramaiah 2009). That cladogram cannot be interpreted in terms of this model without making exceptions at three nodes (minimum) where the total number of spots must have been acquired all at once. A more parsimonious parsing of the data would be to assume that the Nymphalid progenitor had a full set of eyespots.

Together with Wg, Monteiro et al. found Dll in the foci of *B. anynana*, just as Carroll et al. had previously documented for the foci of *P. coenia*. Indeed, it was Dll's presence there that had prompted Carroll et al. to formulate their Flat Leg Model. The new clue that the Monteiro study provided was a timecourse (Fig. 2e). Now, at last, the

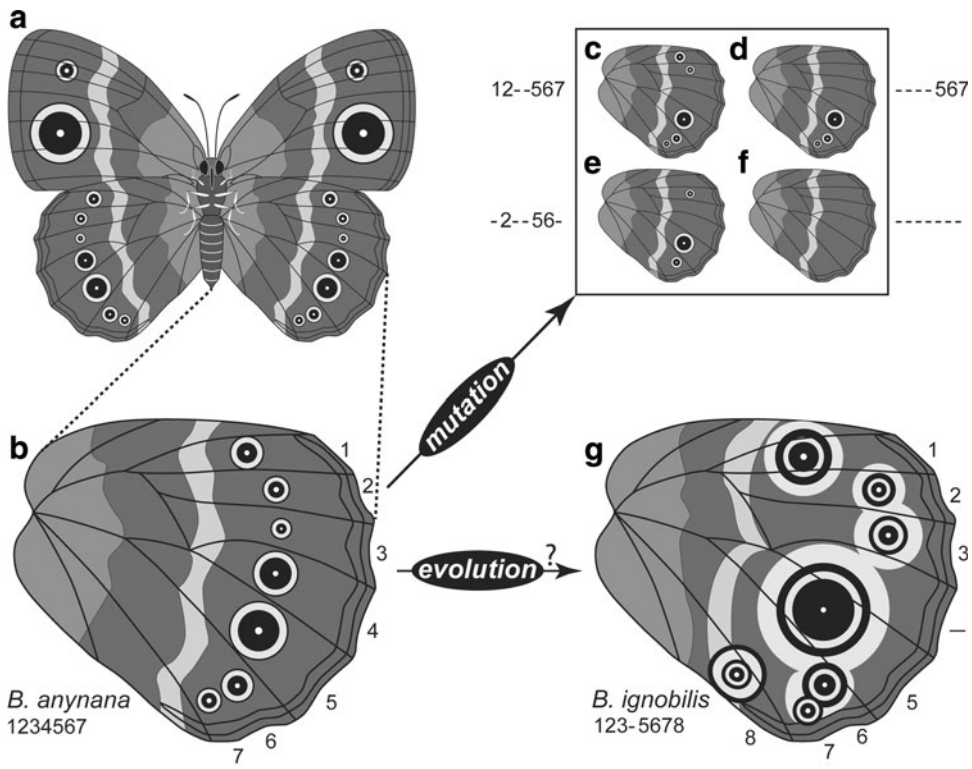


**Fig. 3** Genetics of eyespot patterns. **a** Underside of a wet-season *B. anynana* female (Brakefield et al. 1996). The genus gets its name (*Bicyclus*) from the two big eyespots on the forewing. The forelegs of Nymphalids are vestigial (Wolfe et al. 2010). **b** Enlarged hindwing. The pattern can be represented as a sequence of eyespot numbers (*left*). We do not know how two eyespots (6 and 7) can arise in the same intervein area. **c**–**f** Missing-spot phenotypes recovered after X-ray mutagenesis, labeled as per **b**. Note that spots 3 and 4 are lost jointly. **g** Hindwing of *B. ignobilis*, which presumably evolved from an ancestor whose eyespots were more like those of *B. anynana*—i.e., more uniform. Note the huge eyespot (5), the loss of spot 4, the addition of spot 8, and the extra white outer rings

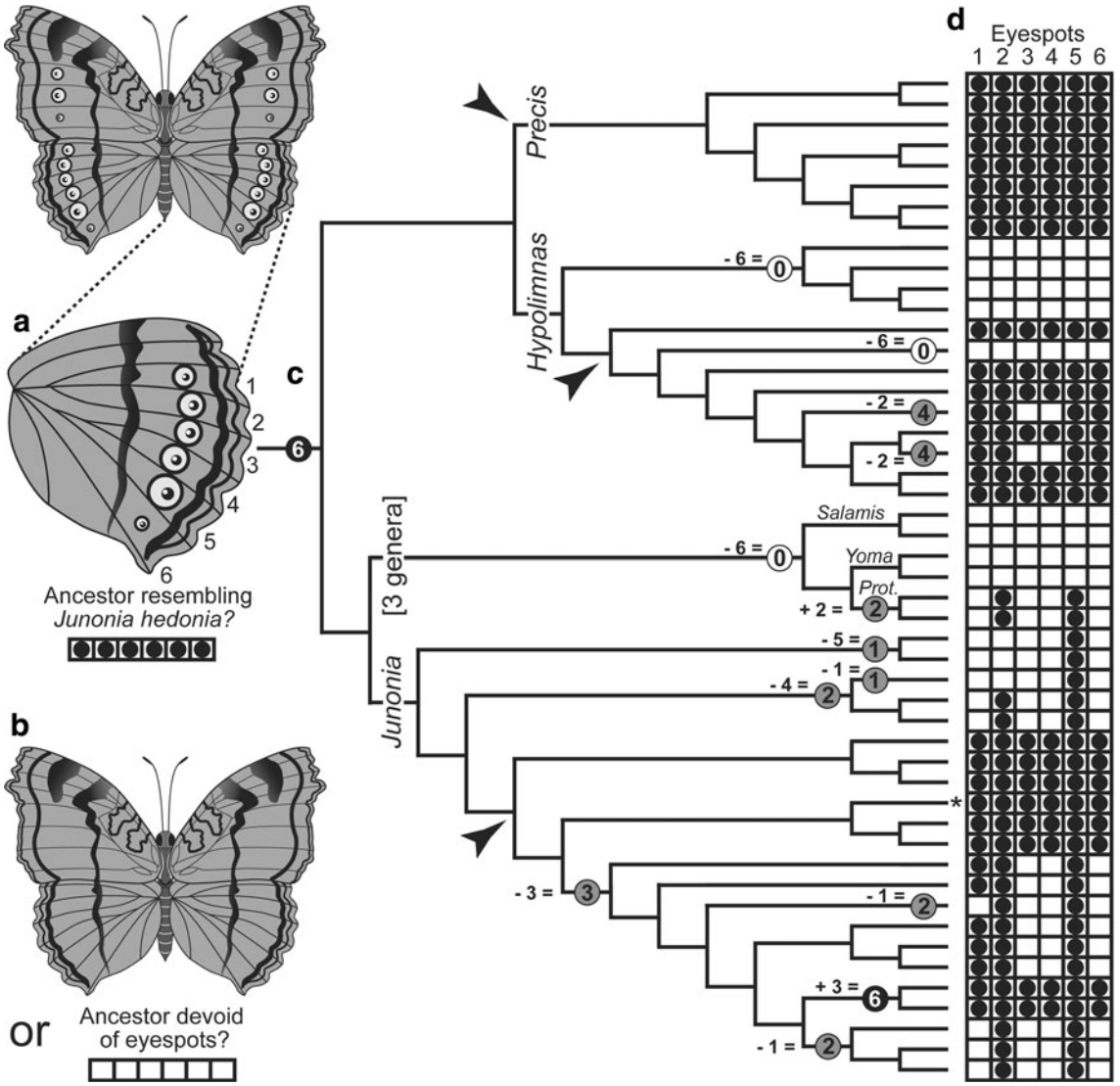


**Fig. 4** Eyespot evolution in Nymphalids. **a** Upper side of *Junonia hedonia*, which is used as a proxy (due to its uniform spot array) for the progenitor of the Junoniini tribe. **b** Spotless hypothetical ancestor (*J. hedonia* sans its spots), which is an alternative prototype. **c** Phylogenetic tree of the Junoniini tribe, whose branches have genera names (*italicized*). *Prot.* denotes *Protogoniomorpha*. *Asterisk* indicates *J. hedonia*. Redrawn from Kodandaramaiah (2009). *Numbers in circles* pertain to eyespots in descendants: *black* (6), *white* (0), *gray* (1–5), with *plusses* and *minuses* denoting change. Numbers were inserted assuming that the ancestor had six eyespots and that lineages lose eyespots more readily than they gain them. Only two gains had to be invoked to fit the data (**d**), and each follows a loss, implying that gains are atavisms, not de novo additions. Alternatively, if the progenitor had no spots, then there are at least three nodes (*arrowheads*) where all six eyespots had to be gained at once. Although possible, this alternative is no more consistent with the Modularity Model (which demands stepwise gains) than the former scheme. **d** Eyespots 3 and 4 are either both present or both absent, as with X-ray mutants (Fig. 3c–f; Monteiro et al. 2003), suggesting a genetic constraint

sequence of gene expression was revealed. Wg precedes Dll by 7 h, which implies that *wg* activates *Dll*, rather than the other way around. Such a link is consistent with the Flat Leg Model because *wg* (in combination with *dpp*) activates *Dll* in the leg (Giorgianni and Mann 2011), but it is equally consistent with the wing margin where *wg* likewise turns ON *Dll* (Zecca et al. 1996; Neumann and Cohen 1997).

Another gem that can be mined from the Monteiro data concerns space, rather than time. Whereas Wg appears to be confined to the focus, Dll is not. Five hours after Dll is detected in the focus its expression expands to fill an area twice the radius of the focus. Dll has a homeodomain, so it is probably not diffusing to this outer limit, though some such proteins can diffuse (Layalle et al. 2001). A more reasonable inference is the *wg* is affecting *Dll* at a distance because Wg itself is diffusing, though the effective level of Wg in the annulus must be too low to detect. This result bolsters the notion that Wg is the focal morphogen.

If eyespots are truly like flat legs, then this model should be easy to prove (Monteiro and Podlaha 2009). To wit, the

nesting of gene expression domains in eyespots should match the nesting of gene expression domains in developing legs (Kojima 2004; Angelini and Kaufman 2005). The ideal gene to assay would be *dachshund*, which is activated directly by *Dll* and turned ON in a ring that overlaps the *Dll*-ON circle in fly leg discs (Giorgianni and Mann 2011). The recent finding that the leg-identity gene *Antennapedia* (*Antp*) is expressed in *B. anynana* eyespots (Saenko et al. 2011) seems to support the Flat Leg Model, but these same authors could not detect any *Antp* expression in *P. coenia*. Hence, the generality of *Antp*'s role is suspect (Castelli-Gair Hombría 2011).

This difference between *B. anynana* and *P. coenia* reminds us that we cannot be sure of Wg's role in eyespots until its presence (and secretion) is documented in the foci of more species than just *B. anynana*. Indeed, firm evidence has already been adduced that a different morphogen—Hedgehog (Hh)—governs *P. coenia* eyespots (Keys et al. 1999), though Hh is secreted from sites that flank the focus rather from the focus itself, making it less likely that Hh is the sole morphogen. Whether Hh acts jointly with Wg or in series with it remains to be determined.

## Eyespots as an Archipelago of Wing Margin Islands?

In Schwanwitsch's iconic map (Fig. 1j) the gap between the submarginal stripes $E_2$ and $E_3$ is roughly equal to the gap between the rings in each of the larger eyespots. This similarity suggests that each focus is analogous to the wing margin, with the eyespot's two circles somehow echoing the margin's two stripes. That metaphor was the impetus for the Recursion Model.

The analogy between foci and the margin might have dawned on us in 1994 when Carroll et al. showed the presence of Dll in eyespots (Carroll et al. 1994), since in this same paper they also reported that Dll is expressed along the wing margin, but instead Carroll's team compared eyespots to legs, where Dll is also expressed. Then in 2006 we missed another chance to notice this connection when foci in *B. anynana* were found to use the same morphogen as the wing margin. We failed to see that Dll's presence could now be reinterpreted as due to a link in the wing's circuitry (*wg* → *Dll*) instead of a genetic link in the leg (*wg* and *dpp* → *Dll*).

Figure 5 sketches how eyespots might have arisen as ectopic copies of the wing margin. Two hypothetical genes are postulated. Gene *A* is expressed midway between wing veins, where foci reside (Fig. 5b), whereas gene *B* is expressed along a perpendicular line that runs parallel to, but at some distance from, the wing margin (Fig. 5a). Eyespots would have originated when a random mutation (at the dawn of the Nymphalids) caused *wg* to be turned ON wherever both *A* and *B* are expressed (Fig. 5c).

For gene *A*, there is one obvious candidate: *Dll*. Before *Dll* is detectable in each focus, it is expressed along a line leading to the future focus from the wing edge (Fig. 2b; French and Monteiro 1994; Carroll 1997). This line might be specified by gradients of EGF, a morphogen made by veins (Fig. 5b; de Celis and Diaz-Benjumea 2003). For gene *B*, there are no suspects at present. However, if Wg can elicit two border stripes, then it is not hard to imagine how it might evoke a stripe of gene expression farther away at a lower concentration ($T_1$ in Fig. 5a). Indeed, Schwanwitsch described just such a stripe in a *Prepona* species: that stripe is pigmented and coincides with the line of foci (Schwanwitsch 1930; his Fig. 9b).

One virtue of the Recursion Model is that it is testable directly. Nymphalids should have a *cis*-enhancer at their *wg* locus that other butterflies lack. We should be able to identify the enhancer by dissecting *wg*'s *cis*-regulatory region (Conceição et al. 2011), coupling each piece to a reporter gene (Marcus et al. 2004), inserting the constructs into host butterflies (Ramos and Monteiro 2007; Fraser 2012), and seeing whether the reporter gene gets expressed in foci (Monteiro and Prudic 2010; Sholtis and Noonan 2010).

This model assumes that eyespots were identical when they first appeared (Fig. 5c), but in modern butterflies the eyespots can vary in size, shape, location, and pigmentation. How did eyespots become independent from one another? The acquisition of independence is termed "individuation," and it has evolved repeatedly in the members of various periodic patterns (Held 2009). The best understood example is insect body segmentation, where different segments were able to diverge evolutionarily ("dissociate") because they express different *Hox* genes (Weatherbee et al. 1998).

How might individuation have occurred in the Nymphalids? Here is where the Modularity Model comes in handy. Monteiro et al. argue that the wing is subdivided into a series of sectors, roughly congruent with intervein regions (Monteiro et al. 2003), within which different transcription factors are expressed. Two of these factors are Engrailed (En) and Cubitus-interruptus (Ci), but the others are unknown, so they are tentatively called C, D, E, F, and G in Fig. 5d. Over time, enhancers might have evolved at the *wg* locus that respond to different gene inputs, enabling certain eyespots to be eliminated (Fig. 5e). Similar enhancers at subordinate genes (for pigmentation, etc.) might have allowed eyespots to specialize in other ways. As a mental exercise, Fig. 5f shows how a big eyespot on the hindwing of *B. ignobilis* (Fig. 3g) might have dissociated from its fellow eyespots.

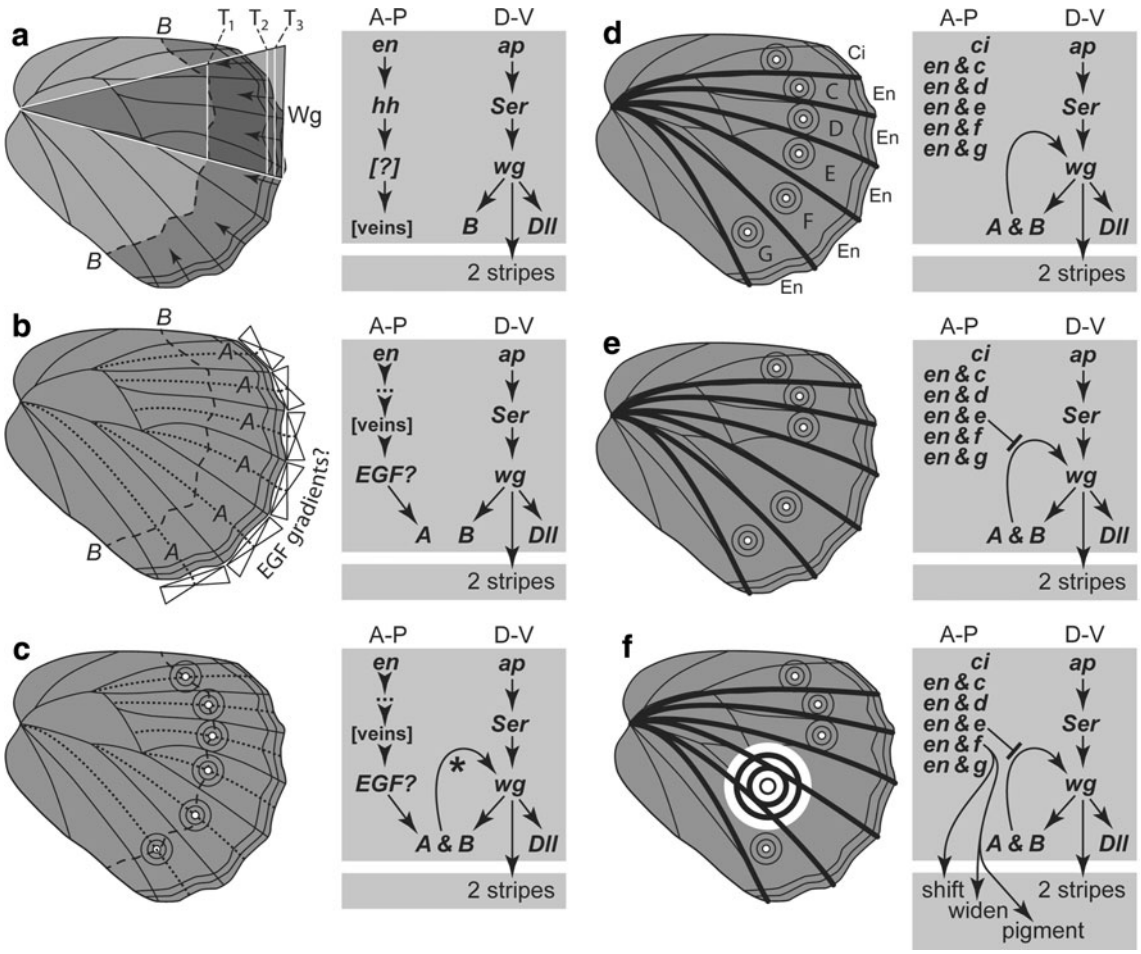


**Fig. 5** Origin and diversification of eyespots. **a–c** Recursion Model, using the ventral left hindwing of *B. anynana* for illustration purposes. *Gray boxes* are hypothetical circuit diagrams (*above*) for the anterior–posterior (A–P) or dorsal–ventral (D–V) axis, or events at the patterning level (*below*). The D/V boundary becomes the distal edge of the adult wing. Circuits are based on flies (Held 2002), but most genes are confirmed in butterflies (Carroll et al. 1994; Keys et al. 1999). *Arrows* indicate activation; *T*-bars denote inhibition. **a** Control of the D–V (proximal–distal) axis by Wingless (Wg). Wg is secreted all along the margin and diffuses proximally (*arrows*) to set up a gradient that is depicted here as a triangle but is more like a rounded ridge tapering to the base. *Distal-less* (*Dll*) is activated by *wg* above threshold $T_1$, with pigment genes being turned ON at levels $T_2$ and $T_3$ to make border stripes ($E_2$ and $E_3$ in Fig. 1j), and an imaginary gene *B* being turned ON at $T_1$ (*dashed line*). Gene abbreviations: *en* engrailed, *hh* hedgehog, *[?]* unknown gene that apparently substitutes for *dpp* in flies, *ap* apterous, *Ser* Serrate, *wg* wingless, *Dll* Distal-less. **b** Metameric control of the A–P axis by a morphogen thought to be EGF (Epidermal Growth Factor) since the EGF homolog "Vein" is emitted by veins in flies (de Celis and Diaz-Benjumea 2003). Triangles denote bidirectional diffusion, with an imaginary gene *A* (*Dll?*) being turned ON at a certain concentration (*dotted line*). The model fails to explain why two eyespots (6 and 7) arise in one intervein sector (Fig. 3b). **c** The event that supposedly created the first eyespots is marked with an *asterisk*. This mutation linked *A* and *B* to *wg*, rebooting the wing margin program (recursively) at every intersection of lines *A* and *B*, leading to (1) secretion of Wg, (2) activation of *Dll*, and (3) specification of two "stripes" that form rings instead (like ripples in a pond) because the Wg source is now a point (focus) instead of a line (margin). To put it crudely, foci (*white dots*) are duped into "hallucinating" that they are at the margin, and they act accordingly. **d** Actual (Ci and En; Keys et al. 1999; Monteiro et al. 2006) and hypothetical (C–G) transcription factors (Monteiro et al. 2003) expressed in sectors of the wing. Even though the eyespots still look alike, they have the ability to diverge because they are "individualized." Each has its own "area code." Adapted from Monteiro et al. (2003). **e**, **f** Hypothetical mutations that may have led to the evolution of an odd eyespot on the *B. ignobilis* hindwing (Fig. 3g). The conversion of covert identities into overt differences, as exemplified here, is called "dissociation." **e** Inhibition of the recursive loop by the combined action of *en* and gene *e* could have erased spot 4, making room for spot 5 to expand? **f** The combined action of *en* and gene *f* on target genes that affect eyespot location, size, and color could have steered spot 5 toward its current garish prominence, though even more elaboration would be required to reach that state. After rogue eyespots became big enough to fool birds into thinking they were real eyes, selective forces would have come into play to enhance the deceptive mimicry even more. The model fails to explain how an eyespot can shift proximally without any shift of the submarginal stripes in its sector since all of these elements should depend on the same Wg gradient slope (Macdonald et al. 2010), though the local threshold for gene *B* might have changed

The Recursion Model contends that eyespots diverged only after the entire series was present. Any subsequent enhancers that evolved at the *wg* locus would have blocked expression of *wg* at certain locations, causing the kind of spot loss that appears to have occurred in some Nymphalid clades (Fig. 4). In contrast, the Modularity Model asserts that eyespots arose one-by-one as position-specific enhancers evolved, so those enhancers should be stimulatory, not inhibitory. Given these different expectations, it should be possible to test them by the same approach described above—namely, assaying potential enhancers using reporter constructs.

## Problems and Prospects

The Recursion Model is no panacea for what ails its predecessors. It has its own embarrassing disabilities. For example, there is the indelicate matter of the endless cycle. Once the genome enters a recursive loop, there is no easy way out except for time to expire. What would a third round of Wg secretion, *Dll* activation, and stripe creation look like? The eyespot's ring of gene *B* expression should intersect the *A* line at two points on the spot's perimeter, leading to two new (fractal) eyespots along that line. Do any butterflies have triplet eyespots aligned between wing veins? (I don't know of any.) Then there is the maddening paradox of what Schwanwitsch called "positional inversion." In the same paper where he shows a pigment stripe intersecting the eyespot chain (Schwanwitsch 1930; his Fig. 9), he describes species where this same stripe ($E_3$ in his groundplan) moved basally beyond the eyespots. That kind of shift should not be permitted if (1) eyespots are specified by gene *B* and (2) gene *B* is activated lower on the wing's Wg gradient than $E_3$.

The outcome of this seemingly quaint contest between clashing conjectures has implications far beyond the butterfly realm. The issue of how evolution tinkers with genomes to invent phenotypes is central to the entire field of evo-devo, and there is no better playground for us to fiddle with models than here in a system with such dazzling diversity (Carroll 1997; Brakefield 2007). Moreover, the simplicity of the geometry is an added bonus. The wing epidermis is as flat as a TV screen and only one cell thick, with no underlying tissues to complicate our analysis. Eyespots offer us a modular microworld with few enough variables that we should be able to tease them apart in the near future.

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