

Butterfly wings: the evolution of development of colour patterns

Paul M. Brakefield^{1*} and Vernon French²

Summary

The diversity in colour patterns on butterfly wings provides great potential for understanding how developmental mechanisms may be modulated in the evolution of adaptive traits. In particular, we discuss concentric eyespot patterns, which have been shown by surgical experiments to be formed in response to signals from a central focus. Seasonal polyphenism shows how alternate phenotypes can develop through environmental sensitivity mediated by ecdysteroid hormones, whereas artificial selection and single gene mutants demonstrate genetic variation influencing the number, shape, size, position, and colour composition of the eyespots. The expression patterns of the regulatory gene *Distal-less* reveal that these changes can arise at several different developmental stages, and the phenotypes indicate that some forms of changed pattern may occur much more readily than others. Further study of the genes, of the developmental mechanisms, and of the functions of the patterns will provide novel insights about the evolution of morphological diversity. *BioEssays* 21:391–401, 1999. © 1999 John Wiley & Sons, Inc.

Introduction

Many studies of the evolution of adaptive traits have provided information on both the genetics underlying phenotypic variation and the basis of differences in fitness among the phenotypes. Usually missing, however, is an understanding of the development of these phenotypic characters and of how the genes could regulate developmental processes to yield the diversity, which can then be sorted by natural selection. Information about developmental mechanisms can lead to predictions about how evolution may be constrained, and whether there is likely to be a bias in the direction followed by evolution. An integrated approach can also provide insights into the mechanisms involved in enabling innovative changes to occur in morphology, giving the potential for adaptive radiation.

The wings of butterflies and moths are decorated with vivid colour patterns that have been shown to function in species recognition, mate choice, camouflage, warning signalling, and in the deflection of predator attack.⁽¹⁾ Mimicry and melanism in the Lepidoptera remain favourite textbook examples of the evolution of adaptive traits.^(2,3) Lepidopteran wings are covered by a fine mosaic of coloured scales that are arranged in a rich variety of patterns. Almost every species has a distinct colour pattern, often showing considerable differences between fore- and hindwings and between the dorsal and ventral wing surfaces. Also, some species are polymorphic, with genetic variants coexisting sympatrically or segregated as geographical races.

In this article, we first examine wing pattern variation in species of butterfly that have evolved phenotypic plasticity in response to alternating seasonal environments. Seasonal polyphenism within a single species is especially useful, both in exploring adaptation and the operation of natural selection, and in examining how one genotype can yield alternate phenotypes through environmental sensitivity and the modification of developmental processes. One colour pattern element that is prominent in seasonal polyphenism shown by some butterflies, is the eyespot, a set of concentric rings of differently-pigmented scales. Recent work has focused on

¹Institute of Evolutionary and Ecological Sciences, Leiden University, 2300 RA Leiden, The Netherlands.

²Institute of Cell, Animal, and Population Biology, Edinburgh University, West Mains Road, Edinburgh EH9 3JT, Scotland.

*Correspondence to: Paul M. Brakefield, Institute of Evolutionary and Ecological Sciences, Leiden University, P.O. Box 9516, 2300 RA Leiden, The Netherlands.

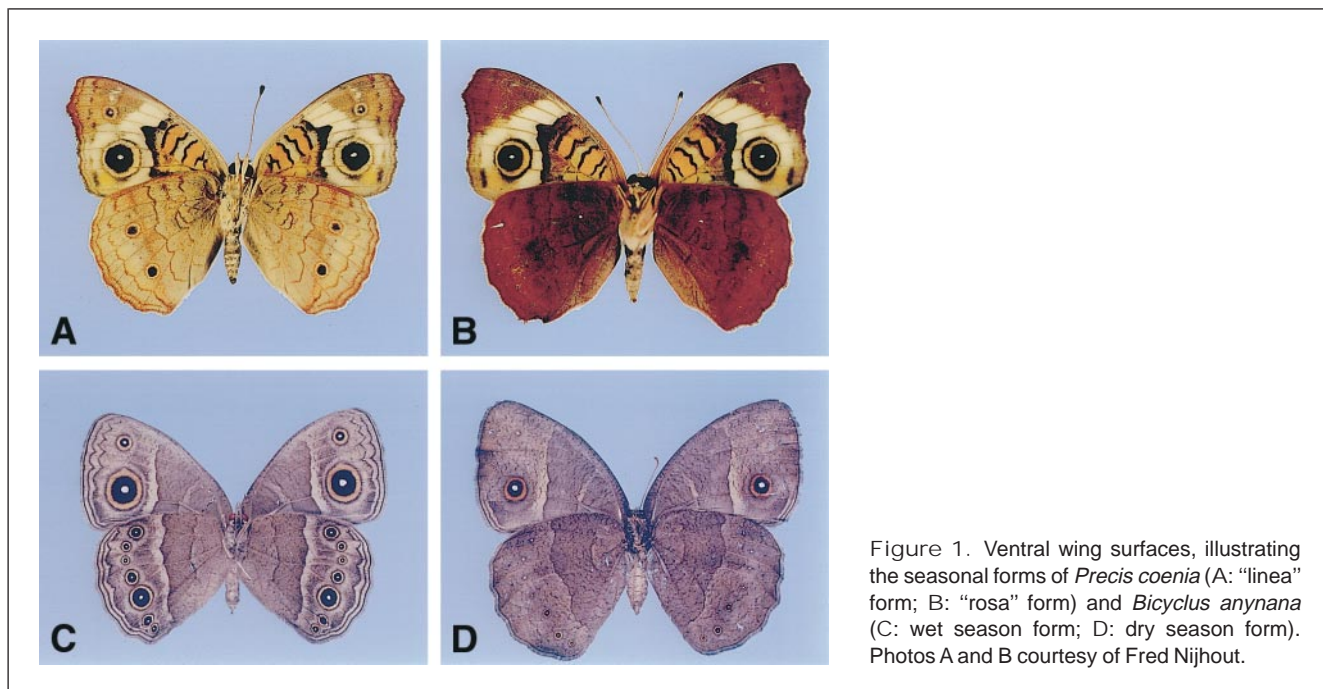


Figure 1. Ventral wing surfaces, illustrating the seasonal forms of *Precis coenia* (A: “linea” form; B: “rosa” form) and *Bicyclus anynana* (C: wet season form; D: dry season form). Photos A and B courtesy of Fred Nijhout.

development of the eyespot and we will examine genetic variation for eyespot patterns and consider how genes may modulate the developmental mechanisms. This leads to examination of the potential for evolution of eyespot diversity, and the relationship between eyespots and the other elements that contribute to the spectacular array of butterfly wing patterns.

Adaptive seasonal polyphenism of wing patterns
 Many species of butterfly are adapted in appearance, behaviour, and physiology to an alternating seasonal environment. Environmental conditions, including the resting background and the nature of predation, can change dramatically through annual cycles of cool-warm or wet-dry climate. Phenotypic plasticity, in the form of seasonal polyphenism in wing pattern, is frequently expressed by multivoltine species with largely non-overlapping generations of adults flying in the alternating seasons.^(4,5) For example, many species of temperate pierid butterflies (the “whites”) show seasonal variability in the extent of wing melanization, with the butterflies that fly in cooler periods of the year having larger black wing markings. In a series of elegant experiments on a North American species, *Pontia occidentalis*, Kingsolver^(6–8) has demonstrated that selection operating on thermoregulation in cool conditions, gives the darker butterflies a higher fitness than the pale summer form. The relative fitnesses are reversed, however, in warmer periods of the year, when the pale form is favoured by selection.

Many species of satyrine butterflies (the “browns”) are adapted to wet-dry seasonal environments in Africa and other

tropical regions.^(5,9–13) Most of these species live close to the ground and are characterized by a wet season form which has conspicuous ventral wing markings, including a ring of marginal eyespots, and a dry season form that has greatly reduced ventral wing markings (Fig. 1C, D). The wet season form is highly active, constantly adjusting its position on a background of luxuriant green foliage. The marginal eyespots and pale medial band on the ventral wing surfaces are visible when the butterflies are at rest with wings raised and closed. The medial band may function to disrupt the outline of the butterfly and the conspicuous eyespots may deflect the attacks of vertebrate predators away from the vulnerable body towards the edge of the wings.^(14,15) The wing edges tear easily and individuals collected in the field often have symmetrical wing damage consistent with this type of (unsuccessful) predator attack.⁽¹⁵⁾ In contrast, butterflies of the dry season form are well camouflaged on a resting background of brown leaf litter and have many months of relative inactivity before reproducing at the beginning of the rains. They lack ventral wing markings, such as the conspicuous marginal eyespots and medial band, which would be likely to attract the attention of insectivorous birds or lizards foraging on the ground. Indeed, in the dry season, released butterflies of the wet season form show markedly lower rates of survival than the resident dry season form individuals (N. Reitsma and P.M. Brakefield, unpublished data). Dry season forms with painted-on eyespots also show reduced survival demonstrating a seasonal difference in fitness directly attributable to the eyespot pattern.

Studies of seasonal polyphenism in butterflies have not only measured natural selection and provided insights into

the basis of the differences in fitness,⁽¹⁶⁾ but are also showing how flexibility in the control of development within a species can lead to the alternative adult phenotypes that match different environments.^(17–19)

Control of seasonal polyphenism

In most studied cases of seasonal polyphenism, the environment affects development primarily through variation in photoperiod, usually interacting with temperature. The North American buckeye butterfly, *Precis coenia*, like all other members of this Nymphalid genus, has seasonal forms that differ in wing colour pattern (Fig. 1A, B). Generations that develop to adult in the summer are of the “linea” form with pale beige ventral hindwings whereas in the “rosa” form, which flies in the autumn, this wing surface is a dark reddish-brown colour. The “linea” form is produced in long-day photoperiods and high temperatures, whereas development of the “rosa” form requires short day lengths.⁽²⁰⁾

The wing pattern polyphenism found in many satyrine butterflies in regions with wet-dry seasons, has been studied in a laboratory stock of *Bicyclus anynana* that was established from a large number of founders collected in Malawi. Larvae reared at high (>23°C) or at low (<19°C) temperatures, produce adults of the wet or dry season form, respectively,⁽¹⁵⁾ whereas variation in photoperiod has no effect in this tropical species.⁽²¹⁾ The final two larval instars are most sensitive to ambient temperature,⁽²²⁾ and the alternative temperature environments in the laboratory broadly match those that occur in the field in Malawi when cohorts of each seasonal form are developing.⁽²¹⁾ The response of the adult wing pattern to different rearing environments is more or less linear with intermediate temperatures giving predominantly intermediate phenotypes (Fig. 2A). Such phenotypes are rather infrequent in the wild,⁽¹¹⁾ where the larvae are likely to be responding to a complex combination of environmental factors. Any factor, such as low mean temperature or poor nutrition, which lengthens larval development enhances the reduction of the eyespots and medial band on the resulting adult wings.^(21–23)

Morphogenetic hormones control many aspects of insect growth and metamorphosis,⁽²⁴⁾ and the ecdysteroids have now been shown to regulate seasonal polyphenism in several species of butterfly.^(19,24,25) Hence the environmental variation in photoperiod or temperature has its effect on the development of the wing colour pattern via the endocrine system. Rountree and Nijhout⁽²⁵⁾ found that *P. coenia* reared under long day lengths and fated to produce the “linea” form, show very low levels of ecdysteroids in early pupae followed by an increasing titer from about 20 h after pupation. In the “rosa” form, however, the increase in hormone levels does not begin until the pupae are 60 h old. Removal of the brain immediately after pupation prevents an early rise in ecdysteroid titer. This operation causes all individuals to develop the “rosa” form, irrespective of their environmental rearing conditions, but the

“linea” form can then be restored in brainless animals by injection of 20-hydroxyecdysone during a sensitive period between 28 and 48 h after pupation.

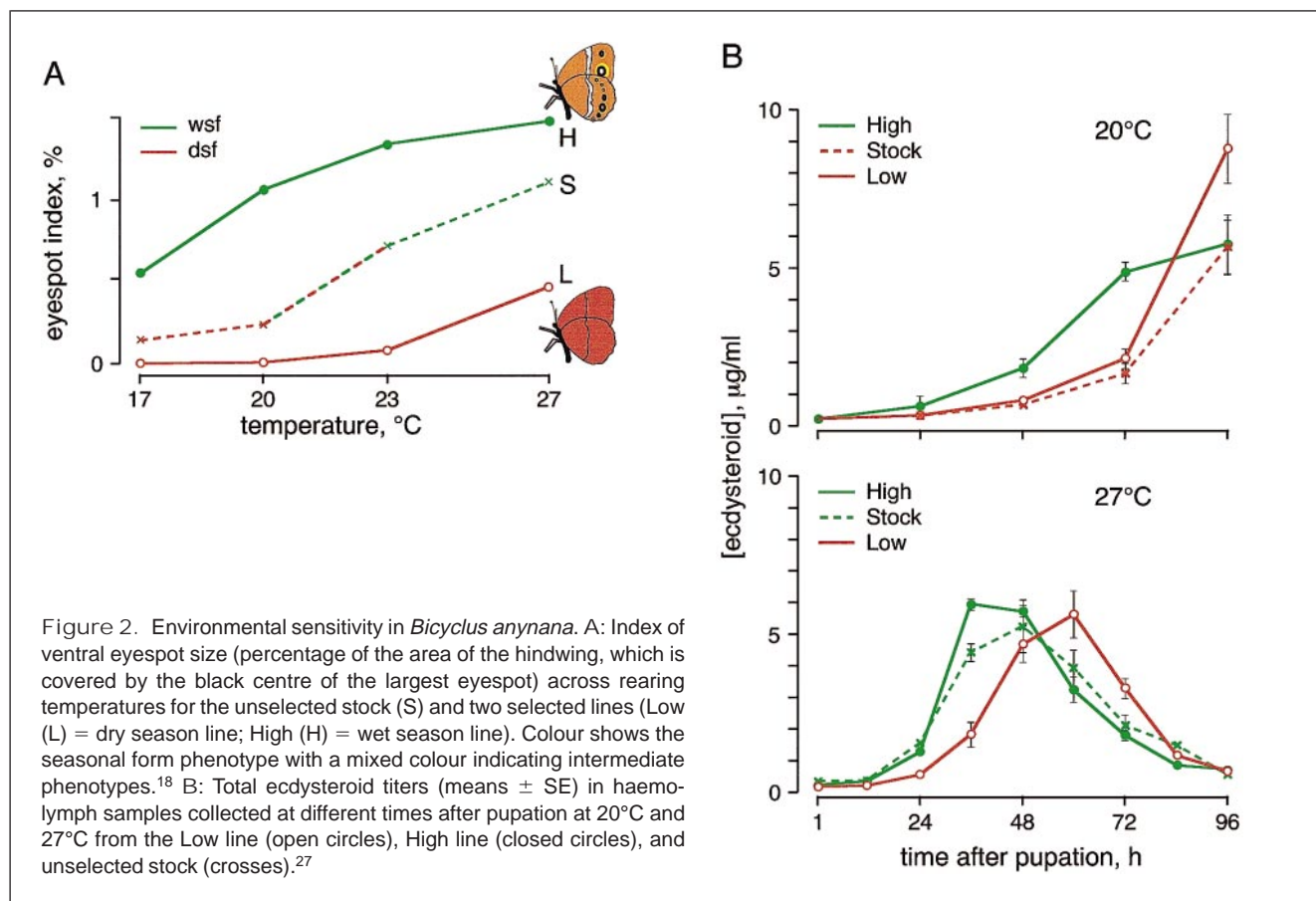
B. anynana naturally responds to rearing temperature but, by artificial selection, separate lines were produced that developed into adults of either the dry or the wet season form at a standard intermediate rearing temperature (described further below). These lines were used to study the role of ecdysteroids in regulating the phenotypic plasticity in ventral eyespots.^(26,27) Ecdysteroid titer rises very early in pupae of the “wet season” line, whereas in the “dry season” line the rise in titer is delayed and more gradual. Furthermore, microinjection or infusion of 20-hydroxyecdysone into very young pupae of the dry season line, results in enlarged ventral eyespots and medial band on the adult wings and thus shifts the pattern towards the wet season form.^(26,27)

Thus these studies in both *P. coenia* and *B. anynana* have demonstrated an important role of the ecdysteroid hormones in the physiological mediation of phenotypic plasticity and seasonal polyphenism in wing colour patterns.

Genetic variation and evolution of phenotypic plasticity

The genetic control of plasticity has been investigated by means of upward and downward selection on ventral eyespot size in *B. anynana* reared at an intermediate temperature.⁽¹⁸⁾ This produced wet season and dry season lines (i.e., High and Low) with highly divergent patterns of response to rearing temperature (Fig. 2A). Wing patterns in butterflies from the wet season line are wet season form at all rearing temperatures, although eyespot size still increases strongly with temperature. The dry season line after selection at progressively higher temperatures now produces only dry season form butterflies (and the reduction in phenotypic plasticity in this line is probably due to the inability of eyespots and medial band to be smaller than zero!). Analysis of the variation in eyespot size found in crosses between the lines suggests that they differ in at least five or six genes.⁽¹⁸⁾

Brakefield and co-workers⁽²⁷⁾ examined ecdysteroid hormone titers in these selected lines, together with the unselected stock, using pupae reared at both low and high temperatures (Fig. 2B). The unselected stock, which switches adult phenotype across these temperatures, shows a shift in hormone dynamics relative to the selected lines. Thus at the low temperature, the stock behaves like the dry season line (and both develop the dry season form of wing pattern), while at the high temperature the stock is comparable to the wet season line, both in terms of a more rapid increase in hormone titer and in the adult wing pattern. This indicates that at least some of the genes contributing to the response to selection on ventral wing pattern, influence the synthesis and release of ecdysteroid hormones.



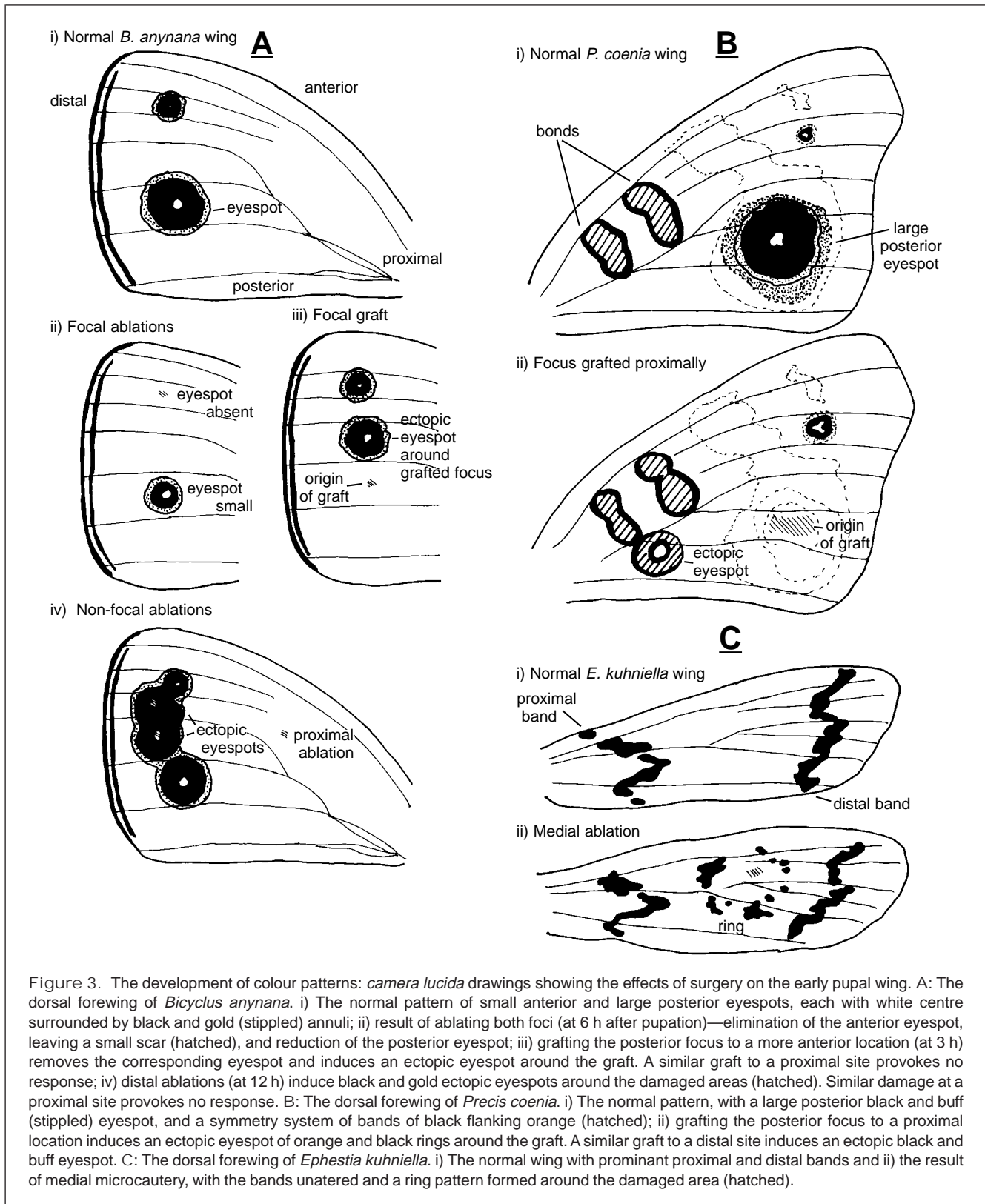
B. anynana has two prominent eyespots on the dorsal surface of the forewing. These do not express phenotypic plasticity, varying very little in size across rearing temperatures,^(13,27) and the microinjections or infusions of 20-hydroxyecdysone into early pupae have no effect on dorsal eyespot size.⁽²⁷⁾ Moreover, lines selected⁽²⁸⁾ for small or large dorsal eyespots (described below) do not differ in the dynamics of ecdysteroid titers following pupation. There is thus an uncoupling between the regulation of eyespot size on the dorsal and ventral wing surfaces, and the ecdysteroid hormones appear to play no role in modulating size during the development of the nonplastic, dorsal eyespots.

Development of eyespots and bands

Lepidopteran wings develop in the larva as two sets of imaginal discs, internal epidermal pouches that evaginate at metamorphosis to form the immobile pupal wings, the forewing overlying the hindwing. The cuticle scales that cover the adult wing are secreted by the dorsal and ventral epidermal cell layers in the late pupa, but cell interactions at earlier stages determine the future colour of these scales and hence define the adult pattern. Wing patterns commonly include two major elements: the eyespots, located midway between wing

veins, and a symmetry system of coloured bands running transversely across the wing (see Fig. 1).

The results of surgical experiments show that the eyespot in both *P. coenia* and *B. anynana* is specified from a central signalling region or “focus” (Fig. 3A, B). Hence damage to the focus in the early pupa removes (or shrinks) the entire eyespot. More critically, transplanting the focus to another position on the early pupal wing induces a concentric eyespot pattern to form ectopically, in the epidermis surrounding the graft^(29–33) and this response may differ dramatically across the wing epidermis (Fig. 3B). Also, on some wing surfaces, local damage to the epidermis can mimic the focal signal, inducing the formation of an ectopic eyespot (Fig. 3A,iv).^(31,32,34) Nijhout⁽²⁹⁾ suggested that the eyespot focus may act as a local source that generates a conical morphogen gradient extending over the surrounding epidermis, with different concentrations specifying concentric rings within which the scale cells later synthesise different pigments. In grafting experiments using foci taken from the anterior (small) or posterior (large) eyespot in *B. anynana*, the size of the induced eyespot depends on the origin of the focus.⁽³³⁾ This result, and the precise shape of fused eyespot doublets (A. Monteiro et al., unpublished), favours a long-range signal,



such as a gradient, rather than a relay of short-range signals initiated by the focus and propagated outwards.

Nijhout^(30,34) has further suggested that a common mechanism may be used to specify the eyespot, the symmetry system of bands, and perhaps other components of wing pattern. Transverse bands, for example, could be specified by a ridge of morphogen resulting from fusion of the gradients generated by a row of foci. The most extensive surgical experiments on band patterns have been done on the pyralid moth, *Ephesia kuhniella*, in which cautery of the early pupal wing can locally deflect a band in ways broadly consistent with disturbance of a gradient profile.^(34,35) There is, however, little evidence for discrete foci⁽³⁶⁾ and, intriguingly, early damage in their predicted location (on the midline of the symmetry system) frequently induces local patterns without affecting the position of the normal bands (Fig. 3C).

If pattern elements, such as eyespots and perhaps bands, are established by signals from discrete foci on the early pupal epidermis, the location of those foci must have been specified earlier, in the larval imaginal disc. There is little experimental evidence, but specification of foci is likely to occur in response to signals from the lacunae that will later form the veins and margin of the pupal and, eventually, the adult wing.⁽³⁰⁾

Gene expression and the wing pattern

Through molecular genetics, much is now understood about developmental mechanisms in the drab little wing of the fruitfly, *Drosophila*. For example, *wingless* and *Decapentaplegic* are expressed in stripes across the imaginal disc and the resulting proteins appear to form morphogen gradients, regulating gene expression and morphological pattern in the proximal-distal and anterior-posterior axes, respectively.^(37–40) Now Carroll and co-workers^(41,42) have shown that many genes are expressed at corresponding locations in the larval imaginal wing discs of both the fly and butterfly (e.g., *Distal-less* and *wingless* distally, *engrailed* and *hedgehog* posteriorly, and *apterous* dorsally). Also, in the butterfly several genes have additional components of expression that suggest roles in specifying colour pattern. Strikingly, *Distal-less* (*Dll*) is expressed in rays between the disc lacunae and then becomes enhanced in small spots corresponding to the foci of the future eyespots (Fig. 4).^(18,41) Also, *hedgehog* is expressed strongly and transiently in patches of cells flanking the focal positions, and expression of the genes *patched*, *cubitus interruptus*, and *engrailed* becomes strongly enhanced in the foci, presumably as a result of *hedgehog* signalling (as has been demonstrated in *Drosophila*).⁽⁴²⁾ These results suggest that genes, and even complex signalling pathways, with conserved roles in development of insect wing pattern (e.g., the veins, sensillae, and wing margin) are deployed again in the butterfly, in specifying the scale colour patterns on the wing surface.

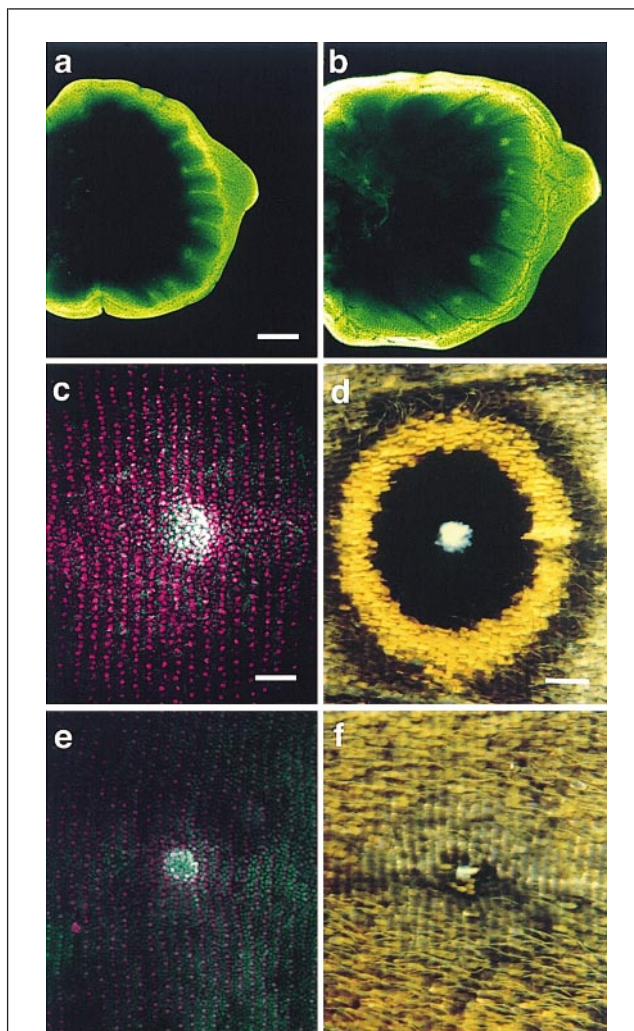


Figure 4. Stages of eyespot formation on the ventral hind-wing of *Bicyclus anynana* as revealed by expression of *Distal-less* (*Dll*). a) and b) show the whole developing wing: a) *Dll* expression in wing disc of mid-final (fifth) instar larvae is highest in the middle of each wing subdivision (bounded by veins); b) by the late fifth instar, the protein accumulates at high levels in the future centre of each of the seven eyespots (cf Fig. 1). c–f) show later development of part of the wing in the two seasonal forms. By 24 h after pupation, scale cells (large nuclei) are aligned into rows and in the wet season form c) *Dll* is expressed in focal epidermal cells and also in scale cells over a large surrounding area corresponding to much of the future eyespot (d). In the dry season form pupa e) *Dll* expression does not spread and no eyespot is formed (f). Scale bars: a) 150 μ m; c) 50 μ m; d) 0.5 mm. Photos kindly supplied by Steve Paddock and Sean Carroll.

Distal-less encodes a putative transcription factor, which is certainly not the focal signal. *Drosophila* mutant phenotypes indicate, however, that *Dll* expression is required for cell interactions in the distal part of the developing leg^(43,44) and

also at the wing margin,⁽⁴⁵⁾ so it may somehow control signalling at the butterfly focus. Whatever the function, *Dll* expression in the butterfly provides a molecular marker for eyespot development. In the larval wing disc, spots of *Dll* expression correspond to eyespot foci: two on the *B. anynana* or *P. coenia* forewing, a ring of seven on the *B. anynana* ventral hindwing, and none in species that lack eyespots.⁽¹⁸⁾ Strikingly, there are no *Dll* spots relating to banding patterns in any of the species examined⁽¹⁸⁾ so, in this respect at least, band and eyespot formation differ in mechanism.

In the developing wing, the changes in *Dll* expression pattern reveal a process that defines the midline of all wing cells and then stabilises as a focus only in specific wing cells (Fig. 4a, b). At around the time of focal signalling, in the early pupal wing, *Dll* expression expands to the scale cells surrounding the focus (Fig. 4c, d). However, in *B. anynana* pupae that are from the dry season selected line, or have been reared at low temperature, this expansion of *Dll* expression from the ventral foci does not occur (Fig. 4e, f). Hence the natural seasonal variation in ventral eyespot pattern, which occurs in *B. anynana* in response to environmental variation in temperature, is mediated by ecdysteroid release and affects the signalling stage of eyespot development on the ventral epidermis of the early pupal wing.⁽¹⁸⁾

Genetic variation and eyespot development

Using a large, outbred laboratory population of *B. anynana*, we have analysed the effects of genetic variation on the development of the wing pattern (Fig. 5). First, we have identified several spontaneous single-gene mutants with major effects on eyespot number, shape, or size. Second, we have used generations of artificial selection to produce lines with eyespots that differ in size, shape, colour composition, or position.

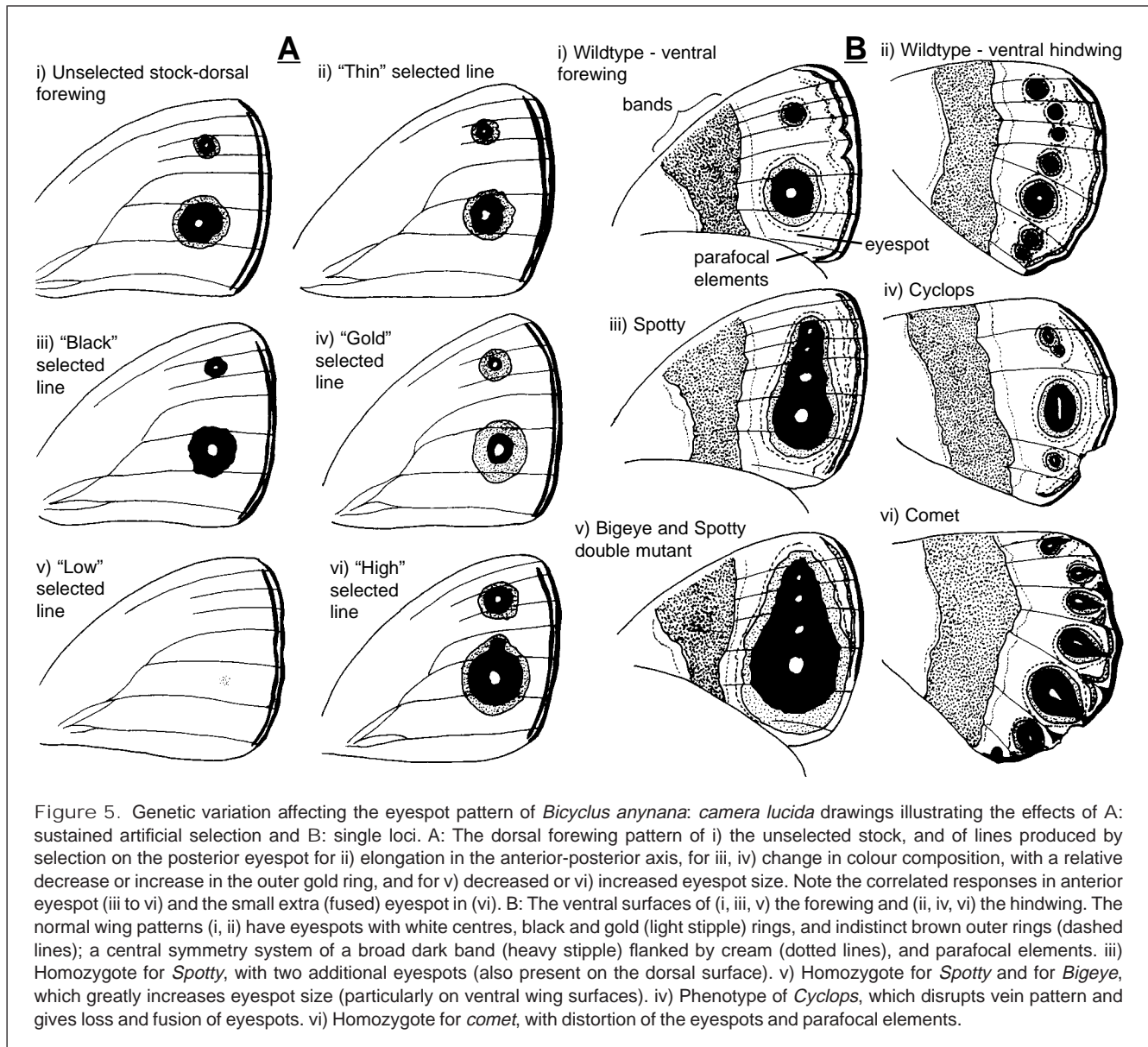
Spotty is a semi-dominant mutation causing the appearance of two additional eyespots on each surface of the forewing, with no effect on other eyespots or the other pattern elements (Fig. 5B,iii).⁽⁴⁶⁾ The *Spotty* forewing imaginal disc shows two additional spots of *Dll* expression, corresponding to the establishment of the extra eyespot foci.⁽¹⁸⁾ The dominant *Cyclops* mutation has a variable phenotype which involves disruptions of the vein pattern with loss and apparent fusion of eyespots, often giving a large, ellipsoidal eyespot on the ventral hindwing (Fig. 5B,iv). In the imaginal disc, *Cyclops* disrupts the lacunae and the midline stripes of *Dll* expression and leads to loss and distortion of *Dll* spots.⁽¹⁸⁾ The *Bigeye* mutation causes enlargement of the eyespots, particularly on the ventral wing surfaces (Fig. 5B,v). *Dll* expression is not altered in the larval and early pupal stages, so it seems that, unlike the other mutants, *Bigeye* affects, not the establishment of foci, but the epidermal response to focal signalling.⁽¹⁸⁾ The recessive mutant, *comet*, produces pear-shaped eyespots which are extended to a variable extent distally along

the midline of the wing cell⁽⁴⁷⁾ and, also, the parafocal elements are distorted and extended proximally along the veins (Fig. 5B,vi). *Dll* expression has not yet been examined so it is not clear which stage of eyespot development is modified by *comet*.

We have also used artificial selection to modify several features of dorsal eyespot pattern in *B. anynana* (Fig. 5A). There was rapid response to selection, in both directions, on the size of the eyespots⁽²⁸⁾ and on their colour composition.⁽⁴⁸⁾ Selection was targeted on the large posterior forewing eyespot, but produced correlated responses in the other eyespots, especially the anterior eyespot on the same wing surface. Clearly there is high additive genetic variance for both size and colour composition, but it appears to influence different aspects of eyespot development. Thus the results of grafting foci reciprocally between pairs of selected lines, indicate that the phenotypic difference in eyespot size (between “high” and “low” lines—Fig. 5A,vi, v) is due largely to genes affecting the focal signals, whereas the difference in colour composition (between “gold” and “black” lines—Fig. 5,iv, iii) results solely from genes that influence the response of surrounding epidermis to the focus.^(28,48) Artificial selection on eyespot shape, however, gave only a limited response, indicating low additive genetic variance.⁽⁴⁹⁾ The slightly elliptical eyespots achieved in the “fat” and “thin” lines (Fig. 5A,ii) do not result from asymmetry in eyespot signalling, but are associated with more widespread changes in wing shape and scale cell distribution.⁽⁵⁰⁾ Thus this phenotypic divergence in eyespot shape may be due to genes affecting growth and expansion of the pupal epidermal sheet after specification of the eyespot pattern. Most recently, artificial selection has yielded a line in which the eyespots are shifted in position towards the wing margins.⁽⁴⁷⁾

In general, selected lines differ from the stock only in the eyespot feature under selection: selection for size has little or no effect on colour composition, and vice versa. Also, there are correlated responses in the other eyespots but, again, only in the feature under selection. Eyespot size and number may represent a partial exception, however, since lines selected for large eyespots show an increased frequency of small “supernumary” eyespots in some wing cells that are normally empty (Fig. 5A,vi).^(28,51) Strikingly, there are no correlated responses in other wing pattern elements (e.g., selection for eyespot size or composition has no detectable effect on the medial band).

The phenotypes resulting from artificial selection, in combination with those from single gene mutation, show that changes in particular features of an eyespot pattern can occur in different ways, both genetically and developmentally.⁽⁴⁷⁾ Thus, both *Bigeye* and combinations of alleles at loci of small phenotypic effect (produced by prolonged selection) can produce similar phenotypes with large eyespots. While the *Bigeye* gene probably modulates epidermal response,



the selected “high” line has accumulated alleles that primarily augment the focal signals. It is also striking that, whereas artificial selection only produces a slow and limited response for eyespot shape, two of the spontaneous mutants (*comet* and *Cyclops*) produce dramatic changes in this feature, probably (in the case of *Cyclops* at least) through changes in the shape of the focus that is established in the larval imaginal disc.⁽⁴⁷⁾

Present deductions about the effects on eyespot formation of single gene mutation or artificial selection, rest on the results of surgical manipulations and on the changes in expression of one gene, *Distal-less*. When the molecular mechanisms of eyespot development are better understood,

it will be possible to study more precisely the ways in which they can respond to genetic change or environmental cues, to produce a variety of phenotypes and, thus, the basis for evolution of diversity.

Evolution of wing patterns

In most lepidopteran species, the wing patterns are complex, often consisting of sets of eyespots, bands, and other elements, serially repeated in all or some of the wing cells (Fig. 1C). Nijhout and his colleagues have emphasized the way in which the independent development of different pattern elements has contributed to the evolution of the spectacular diversity of wing patterns. They propose that

morphological change within a particular set of pattern elements can proceed more or less uncoupled from change in the other sets of elements.^(17,30,52–54) Analyses of both phenotypic and genetic correlations among traits on the wings of *P. coenia* and *P. evarete* illustrate the modularity within the complete wing pattern. Thus the correlations are consistently stronger among sets of eyespots or other characters than between such sets.^(52–55) This predicts that the separate sets or series of developmental homologues are free to follow their own evolutionary trajectories as dictated by specific functional requirements of natural selection. However, evolutionary change within any one of these sets (e.g., between the different eyespots), is likely to be more constrained by common developmental mechanisms and high levels of genetic covariance.^(46,47)

The developmental and genetical studies of the eyespot pattern of *B. anynana* also indicate that evolutionary change is likely to be strongly constrained within a set of pattern elements (Fig. 5). Thus, some mutations such as *comet* and *Bigeye* have effects on the colour pattern that are largely restricted to the eyespots and are comparable on all eyespots. Similarly, artificial selection on features of a particular eyespot does not influence other elements, but does produce similar (although less extreme) changes in the other eyespots.

These results suggest that certain forms of change in the wing pattern are comparatively open to adaptive evolution whereas others will be much more strongly resistant. The positively correlated responses suggest that it may be relatively difficult for change in one feature (e.g., size or colour composition) to occur only in some eyespots, or for changes in opposite directions to occur in different subsets. These predictions will be tested by appropriate artificial selection experiments.⁽⁴⁷⁾ Our own studies of mutant eyespot phenotypes in *B. anynana* suggest that certain genes (e.g., *Spotty*), which have a localised effect on subsets of eyespots, may uncouple developmental homologues and facilitate novel evolutionary changes. A challenge for the future will be to understand both the modes of developmental action of such genes and their roles in the observed diversity across species. In the genus *Bicyclus*, for example, there are species with eyespots atypical in number or in relative sizes or proximal-distal positions.^(46,47) Also, it is notable that in some species, including *B. anynana*, the dorsal and ventral eyespots are uncoupled in their response to environmental cues.^{27,55}

In considering the development and evolution of wing pattern, we have featured work on species with simple eyespots. There are also several well-studied species with rather different colour markings that are highly polymorphic and have been shown to function in warning and mimicry.^(56–61) For example, *Heliconius erato* and *H. melpomene* are members of a ring of Mullerian mimics and the striking differences

in colour pattern between any two of their numerous geographical races are usually due to allele differences at only four or five gene loci.^(57–60) Comparative analysis of wing patterns in the genus *Heliconius* indicates that the distinctive markings may lie between black pattern elements, predicts locations for the foci, and also suggests how genetic differences may influence the position and/or activity of these foci.^(62–63) If patterning foci can be directly demonstrated by surgery or gene expression patterns and, particularly, if the gene loci become amenable to molecular study, then *Heliconius* will become an exciting system for studying the evolution of wing pattern.^(46,64)

Conclusions

Butterfly wing patterns frequently show spectacular differences, among closely related species and even among individuals of the same species. For butterflies, the wing pattern is an important functional component of the phenotype, subject to natural selection in the context of interactions with predators, with the resting background, with potential mates, or with the abiotic environment.

Colour pattern elements develop in a single layer of epidermal cells, and transplant and damage experiments have demonstrated that the concentric colour rings of an eyespot are specified around a signalling focus. Studies of homologues of genes known from *Drosophila* wing development (notably *Distal-less*) have provided insights into the stages of eyespot formation. This approach, augmented perhaps by study of other genes identified directly through their localized expression or mutant phenotypes, should lead eventually to an understanding of focal signalling and of the earlier specification of the eyespot foci on the wing epidermis. In addition, it should become clear to what extent the other elements of wing pattern resemble the eyespots in their developmental mechanism.

The ways in which pattern diversity can be generated within species have been investigated through seasonal polyphenisms, in which phenotypic plasticity occurs in response to variation in some environmental factor during pre-adult stages. In several species, the environmental effect is mediated by changing titers of ecdysteroid hormones and future work should establish how this regulates the development of specific pattern elements. The effects of genetic variation have also been studied, notably on the eyespot elements of the butterfly *B. anynana*. Surveys have been made both of alleles of single genes, which produce an abrupt change in eyespot number, size, or shape, and of the effects of artificial selection on multiple genes of small phenotypic effect, producing gradual changes in eyespot size, colour composition, shape, or position. The use of surgical manipulations and the study of *Distal-less* expression in the eyespot mutants and selected lines has already shown that changes can arise at several different stages in eyespot development.

Furthermore, it is becoming clear that some types of change in eyespot pattern may occur much more readily than others.

When combined with phylogenetic analysis of morphological divergence within a group of species (e.g., the species-rich genus *Bicyclus*) this synthesis of genetics and development may begin to show how constraints, arising from the context of gene function within developmental processes, can contribute to bias in the trajectories of evolution. Butterflies will clearly continue to provide the basis for integrative research on evolution—from the selection pressures, to genetic differences, to the molecular mechanisms generating differences in development and thus in the phenotype.

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