THE CHARACTER CONCEPT IN EVOLUTIONARY BIOLOGY

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THE STRUCTURE OF A CHARACTER AND THE EVOLUTION OF PATTERNS

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INTRODUCTION: DISSECTING A CHARACTER

This essay examines concepts about character evolution in the context of genetical and developmental studies of a specific morphological pattern, that of butterfly eyespots. Although semantics may differ, many of the underlying ideas have already been expressed by Müller and Wagner (1991), Wagner and Altenberg (1996), and others, but here the discussion stems from a single model system. The eyespot pattern of butterflies is composed of a series of individual eyespots or units. Morphological descriptions suggest that the most appropriate and useful application of the term "character" is to the complete eyespot pattern or, perhaps, to the eyespots on a specific wing surface.

I will focus here on several issues based on an analysis of variation in a single species of satyrine butterfly, *Bicyclus anynana*. Our approach centers on the internal genetical and developmental organization of the eyespot pattern (for reviews, see French, 1997; Brakefield, 1998; Brakefield and French, 1999). It has begun to examine how flexibly and rapidly different units or features of the trait may be able to respond to change in the environment and natural selection.

Such an integrated study may reveal the extent to which estimating genetic variances and covariances provides a satisfactory description of character structure. An understanding of how genes modulate development and what genetic variance-covariance matrices reflect in mechanistic terms seem likely to reveal additional insights about character structure and, therefore, about the potential for evolutionary change. A description of the genes and of their developmental roles will also be crucial to formulating predictive models of eyespot formation (see Nijhout and Paulsen, 1997). A knowledge of the internal organization of a character should also reveal whether bias is likely in the production of novel phenotypes, and how such bias relates to the developmental processes. Bias may have resulted in patterns of morphological diversity in eyespot patterns which have been shaped by some balance between natural selection and developmental constraints (see Maynard-Smith *et al.*, 1985; Schlichting and Pigliucci, 1997). Comparisons of such predictions about the likelihood of evolutionary trajectories with observed patterns of change across related taxa should then enable the robustness of ideas about character evolution to be tested.

In investigating the evolution of an eyespot pattern using a multidisciplinary approach we will eventually be able to examine whether the ways in which genes modulate development influence their probability of becoming involved in evolutionary divergence. Putting this point another way, we can examine whether when there are alternative ways in which development can be modulated to produce a given phenotype, can predictions be made about their relative contribution to evolution? Furthermore, studying both the genetics and development of a character should provide insights about whether there is something special about abrupt changes or shifts in phenotype; are such events more likely to involve developmental novelties or additions to the developmental repertoire?

EYESPOTS COMPRISING A MORPHOLOGICAL PATTERN

Ten years ago we began to study the evolution of the wing pattern of African *Bicyclus* butterflies. The most prominent wing pattern element in these butterflies is the marginal eyespot (Fig. 1). These eyespots are frequently exposed while butterflies are at rest. They are likely to function in avoidance of vertebrate predators which hunt their prey by sight. They can act as targets deflecting predator attacks away from the vulnerable body. In this way, they can help the butterfly to escape, albeit having lost part of the outer wing tissue (Wourms and Wasserman, 1985; Brakefield and Reitsma, 1991).

FIGURE 1 A single ventral eyespot of *B. anynana* showing the concentric rings of color. Individual overlapping scale cells can be seen in the gold ring.

Each eyespot consists of a series of annuli arranged around a central white pupil (Fig. 1). Each concentric ring is itself composed of many individual scale cells which contain the same color pigment. The pigments are synthesized toward the end of the pupal stage, shortly before adult eclosion. The different colours of an eyespot are thus produced by sharp transitions in the pigments across the boundaries between the rings. The scale cells are arranged across the wing blades like the tiles on a roof. Each wing surface is effectively a single layer of epidermal cells, some of which are differentiated into the pigmented scale cells (Galant *et al.*, 1998). The two innermost rings of an eyespot in *Bicyclus* are black and gold. Some ventral eyespots may have further rings of different colors (see, e.g., Fig. 1).

Most of the research on *Bicyclus* eyespots has used a laboratory stock of *B. anynana* established from a large number of founders from a single locality in Malawi. The species is easy to rear in large numbers on maize and has a short generation time. *B. anynana* can have eyespots on all four wing surfaces, although they are often absent on the dorsal hindwing (Fig. 2). The wings of a butterfly are bisected by strengthening wing veins or trachea, most of which run proximal to distal. Each wing subdivision, which is bordered by wing veins, is known as a wing cell. The ventral hindwing in *B. anynana* usually shows a complete series of eyespots, one in each wing cell along the wing margins. The

eyespot are aligned along the fold or internervule running midway between each pair of veins (Fig. 2A). In contrast, both surfaces of the forewing normally express only two eyespots: one anterior and small and the other posterior and large. Eyespots are usually absent from the two intervening wing cells, as well as from those which lie more anteriorly or posteriorly.

Comparative morphologists in the 1920s and 30s were concerned with describing patterns of wing pattern diversity across species within different major groups of the Lepidoptera, including the Nymphalidae (for review see Nijhout, 1991). Perhaps their most important contribution was the recognition of a so-called prototype or ancestral wing pattern which shows a full ring of marginal eyespot elements on each wing surface. This prototype also incorporates other pattern elements, including medial and parafocal band markings. Some changes in eyespot pattern within the Nymphalidae (which includes the Satyrinae) are then envisaged as involving reductions in eyespot number by their loss in one or more wing cells. Certain extant species appear close to the prototype pattern with a complete complement of eyespots, while many others show no distinct eyespots (see Nijhout, 1991, for illustrations). From the perspective of comparative morphology, the whole eyespot pattern appears to correspond to a single character or module which itself comprises a series of repeated eyespot elements. There is a potential for an eyespot to be expressed in each marginal wing cell. This confers an identity on each eyespot in terms of its position, and enables comparisons of patterns of presence and absence across individuals or taxa.

FIGURE 2 The typical eyespot pattern on each wing surface in *B. anynana*. All wings are from the wet season form using representative specimens of the unselected stock reared at 27°C.

This type of morphological organization is supported by a more statistical approach for individual species to examine phenotypic correlations among the eyespots. In another satyrine butterfly, *Maniola jurtina* (Brakefield, 1984), and in the nymphalids, *Precis coenia* and *P. evarete* (Paulsen and Nijhout, 1993), the size of any particular eyespot is positively correlated with the size of other eyespots, especially those on the same wing surface.

If the concept of the prototype is valid it becomes interesting to ask how the pattern of the character it represents can be changed through evolution to yield the present set of states, including those found within *Bicyclus*. Descriptions of genetic variances and covariances, together with information about developmental mechanisms, are also necessary to discover how the eyespot pattern can be decomposed into units or modules which have a (higher) degree of individuality in their development, and thus in their potential for independent evolutionary responses to selection. With such information, we can also eventually match the observed set of patterns to those which our analysis of the internal structure of the character predicts are more likely to be readily generated at the phenotypic level.

DEVELOPMENTAL MECHANISMS AND MORPHOLOGICAL ORGANIZATION

Working with the large posterior eyespot on the forewings of the buckeye butterfly, *Precis coenia*, Nijhout (1980) made the breakthrough which was crucial to revealing the developmental mechanisms of eyespot formation. Butterfly wings develop in the late larva as paired internal epidermal pouches, the imaginal discs. There are two discs on each side of the insect. These protrude at metamorphosis to form the pupal wings, the forewing overlying the hindwing. The cell layer of the dorsal forewing lies immediately under the pupal cuticle, and for a few hours after pupation it is attached to the cuticle. The critical events in pattern determination occur in the early pupae. The trachea of the forewing are visible through the pupal wing case. There are also pupal markings or raised areas of cuticle which overlie the central regions of putative adult eyespots. These provide landmarks for experimental manipulation of eyespot formation.

First, Nijhout (1980) used cautery with fine needles inserted through the pupal cuticle to damage cells of the developing forewing. He found that damage to cells in the central region of the putative posterior eyespot could produce dramatic reductions in the eventual size of the eyespot; the earlier the damage, the smaller the eyespot. More critically, shortly after pupation he was able to transplant the cells of the central region to an adjacent area of the wing where no eyespot pattern was normally observed. An ectopic eyespot formed around the grafted tissue.

FIGURE 3 A. An ectopic eyespot produced after transplantation of the focus of the posterior eyespot. B. Diagram of the developmental pathway of eyespot formation.

These results have been fully substantiated by subsequent experiments on *B. anynana* (French and Brakefield, 1992, 1995; Brakefield and French, 1995, 1999). Figure 3 illustrates an example of the result of transplanting an eyespot focus in this species. The overall results are also consistent with Nijhout's (1978) model of eyespot formation in which the focus induces a signal, presumably by diffusion, to surrounding cells. This results in a cone-shaped concentration gradient. At the end of signal induction the surrounding cells

"interpret" the gradient, thus gaining information on their position relative to the central focal region and becoming fated to synthesize a particular color pigment.

The later experiments on *B. anynana* have, however, provided several additional insights. First, damage experiments to nonfocal areas of the distal wing blade performed shortly before the end of pattern determination (ca. 24 h after pupation at 27°C) can produce ectopic eyespots which are closely similar to control eyespots except that they have no central, white pupils (cf. in *P. coenia*, Nijhout, 1985). Perhaps, the eyespot foci act as sinks rather than sources. A uniform field of morphogen is then viewed as being initially present across the wing (French and Brakefield, 1992). This is degraded around active foci in early pupae, producing sink-like troughs in the profile. Damage shortly after pupation would then initially yield sinks but later diffusion and healing would tend to recreate the uniform morphogen profile. On the other hand, damage shortly before the end of pattern determination would produce similar sinks but without the time for healing by diffusion from surrounding cells. Results of series of damage experiments, which included the use of different severities of damage, are broadly consistent with this type of interpretation but there are no data which unambiguously distinguish between a source or sink model (French and Brakefield, 1992; Brakefield and French, 1995).

Second, experiments in which cells are damaged in the proximal (inner) region of the forewing do not produce ectopic patterns (Brakefield and French, 1995). Furthermore, focal grafts made into this region of the wing also yield no ectopic patterns. Additional transplantation experiments indicated that while signal transduction can occur through the cell layer of the proximal part of the wing, the cells are not competent to respond, or cannot synthesise a pigment other than that of the background, brown color (French and Brakefield, 1995). Similar distal to proximal grafts performed in *P. coenia* produce a dramatic difference in the ectopic color pattern. While grafts made to distal positions give patterns with a white outer eyespot ring, those to a proximal position yield an outer ring with the same color as two prominent orange bands which occur in that area of the forewing (Brakefield *et al.*, 1996). Variation between scale cells in their competence to produce specific color pigments must be one important mechanism generating spatial color variation in many butterfly wings, as well as differences among individuals and species (see also Koch *et al.*, 1998).

Carroll *et al.* (1994) showed that the developmental gene, *Distal-less*, is expressed in early butterfly wing discs in rays midway between the lacunae that will form wing veins. Later, shortly before pupation, *Distal-less* expression in the rays degrades but their proximal tips become enhanced in small circular groups of cells which correspond to the foci of the future eyespots. The use of *Distal-less* as a molecular probe has enabled a fuller description of eyespot formation in *P. coenia* and *B. anynana* in the form of a developmental pathway (Brakefield *et al.*, 1996). This can be viewed as involving four stages of

regulation which are in sequence: (1) establishment of a prepattern with potential foci; (2) determination of the foci; (3) signaling from a focus induces surrounding cells; and (4) induced cells later differentiate into scales of different colors depending on their distance from the focus and their position in the wing. Most recently, Keys *et al.* (1999) have shown that other developmental genes which are involved in wing formation in *Drosophila* have additional components of expression in butterflies that suggest roles in specifying the eyespot pattern. For example, *hedgehog* is expressed strongly and transiently in areas flanking the eyespot foci while the genes *patched*, *cubitus interruptus*, and *engrailed* become strongly enhanced in the foci themselves, presumably as a result of hedgehog signaling. Further advances in this research at the molecular level are likely to reveal precisely how such signaling pathways with conserved roles in insect wing development are deployed in butterflies in specifying the wing color patterns. We also hope to be able to match some eyespot patterning genes to the regulation of specific signaling pathways.

Thus, this collaborative research effort has provided a basic understanding of eyespot development, although most of the details remain unclear. The involvement of a morphogen diffusion gradient rather than some form of cell-cell relay system has not been demonstrated unambiguously. One important interpretation arising from all these studies is that eyespot formation involves a developmental mechanism which is common to all eyespots on butterfly wings. There is thus convincing support for the concept that the different eyespots are serial repeats and for the view from comparative morphology that the eyespot pattern as a whole represents the most appropriate choice for the unit character. In this context, it then becomes critical to examine potential mechanisms by which differentiation can evolve among the eyespots corresponding to some prototype pattern. We have begun to examine this issue by concentrating on genetic variation for the eyespot pattern in *B. anynana*.

GENETIC VARIATION AND DEVELOPMENT OF FEATURES OF THE EYESPOT PATTERN

High phenotypic correlations are found among the individual eyespots of species including within the genera, *Bicyclus*, *Maniola*, and *Precis*. These are associated with positive genetic correlations (Brakefield and van Noordwijk, 1985; Holloway *et al.*, 1993; Monteiro *et al.*, 1994; Paulsen, 1994, 1996). This is unsurprising in view of the common developmental mechanism of eyespot formation. The issues of how evolutionary divergence occurs can then be considered in terms of either developmental or genetical mechanisms. Among the specific questions which can be posed from the former perspective are: how can development be uncoupled across eyespots, and how do novelties and modifications of the developmental pathway occur? The parallel questions in terms of genetics then become: how can genetic correlations be changed, and

what is the pool of mutants available for genes with localized positional effects and novel phenotypic changes? Linking development and genetics might then indicate how bias in the generation of phenotypic variation is related to developmental organisation. We have tried to integrate the genetics of phenotypic variation in *Bicyclus* eyespots with the developmental insights.

Taking as a character the eyespot pattern based on repeated units of single eyespots, we can then also consider different features or aspects of the pattern and its constituent units. We have recognized the following four features: overall size, color composition, shape, and position. In addition, we have some information on both eyespot number and the presence or absence of particular eyespots. Several years ago we began a series of artificial selection experiments to explore genetic variances for different features of the eyespot pattern of *B. anynana* (Monteiro *et al.*, 1994, 1997a,b,c; Brakefield, 1998). We have also established a series of spontaneous single gene mutants which show a variety of effects on the eyespot pattern (Brakefield *et al.*, 1996; Brakefield, 1998; Brakefield and French, 1999). Our observations indicate that many genes are involved in eyespot formation. Future research will use mutagenesis and QTL gene mapping to more intensively probe the genetics. The best way to illustrate the findings to date and to expand on how such results should yield sounder predictions about evolutionary trajectories for the eyespot pattern is to make specific contrasts among the different features, or among the different mechanisms of producing similar phenotypes.

A. Eyespot Size and Color

Upward and downward artificial selection has been applied to each feature of the large posterior eyespot on the dorsal forewing of *B. anynana*. Lines were obtained with large or small (ultimately absent) eyespots, or with "black" or "gold" eyespots (the outer gold ring either being very narrow or broad relative to the inner part of the eyespot). Fully divergent phenotypic distributions were obtained within five to ten generations (Monteiro *et al.*, 1994, 1997a). These included phenotypes not represented within the unselected stock population (Fig. 4). Although selection was only directed at the posterior eyespot, other eyespots, especially the anterior forewing eyespot on the dorsal surface, tended to show concordant responses to selection. Thus, as mentioned earlier, there are positive genetic correlations among eyespots. Realized heritabilities were slightly higher for eyespot size. At around 50%, they were comparable with morphological characters in *Drosophila*, and they indicate substantial additive genetic variance. The rapid and prolonged responses to selection were consistent with contributions of a number of genes, each of small phenotypic effect. In contrast to the positive genetic correlation for a particular feature among eyespots, correlated responses between eyespot features were minimal (see further later). This is especially striking in specimens from the "black" and "gold" selected lines (Fig. 4).

FIGURE 5 Four mutant phenotypes of *B. anynana*: A, *cyclops*; B, *Spotty*; C, *comet*; and D, *Bigeye*. The ventral wing surfaces are shown of female individuals (dorsal forewing patterns tend to follow those of the ventral surface). Each specimen is homozygote for the *Spotty* allele (the wild type is shown in Fig. 2).

Backcrossing the *Bigeye* allele into the downward Low-line for eyespot size for six generations has yielded a line in which *Bigeye* heterozygotes can only be detected by their ventral phenotype (unpublished data). The dorsal eyespots are very small or absent. This is consistent with an effective absence of focal signals in the Low line combined with an effect of *Bigeye* at the level of the response to this signal. In contrast, backcrossing the *Bigeye* allele into the upward High-line appears to yield an additive phenotypic effect in which the resulting eyespots can be larger than in the original *Bigeye* stock. Again, this is predictable from the interpreted changes in development in both the stock and the selected line. Combining *Bigeye* with *Spotty* increases the size of the novel eyespots (Fig. 5).

The occurrence of *Bigeye* and the responses to selection on eyespot size show that similar phenotypes can be produced by very different genetical and developmental changes. A further contrast is illustrated by the lines for eyespot size and color. Although the additive genetic variance is similar for these features, the underlying developmental changes are different. These observations raise a number of issues which are as yet unresolved (Brakefield, 1998). First, for a given change in a feature of a character, does it matter in terms of likelihood of involvement in the evolution of divergence whether a single gene or a number of genes is involved? Second, if two characters or features of a character behave similarly in terms of rate of response to directional selection, does it matter whether they have a similar or dissimilar developmental basis?

B. Eyespot Shape

The eyespots of *B. anynana* have a circular shape. Artificial selection on eyespot shape (toward "fat" or "thin") produced a much slower response than observed for the other features (Monteiro *et al.*, 1997b, c). Realized heritabilities were only about 15%. There were also indications of a rather rapid approach to a limit for the response to selection. Morphometric studies showed that the response to selection was at least partly accounted for by changes in wing shape and in the matrix of scale cells around the forewing eyespot.

Perhaps the options for changing eyespot shape in a bilaterally symmetrical way through modifying the basic components of the developmental pathway of eyespot formation are more limited than for other features (it would be interesting to pursue such ideas by theoretical models, cf. Nijhout and Paulsen, 1997). However, abrupt effects are possible as is shown by two of our mutants, *cyclops* and *comet*, which dramatically change eyespot shape (Fig. 5). The fundamental developmental effect of the former mutant is a change in the venation pattern; one of the major veins is vestigial in each adult wing, leading to a fusion of the flanking eyespots (Brakefield *et al.*, 1996). However, such

pleiotropic effects of venation mutants on wing pattern may not be very important in evolution. *Cyclops* is homozygous lethal, and the heterozygotes of mutant phenotype rapidly damage their wings following eclosion, presumably as a direct consequence of the change in venation.

Comet is a fascinating allele for several reasons. Homozygotes not only have the pear-shaped eyespots but the pattern of the parafoveal elements (border chevrons and bands) is disrupted producing proximal extensions along the wing veins. Most strikingly, males of this genotype have highly vestigial secondary sexual characters (V. Schneider and P.M. Brakefield, in preparation). Wild-type male *B. anynana* have highly developed androconia in the proximal region of the dorsal hindwing. These comprise two plumes of long hair-like scales overlying regions of other modified scales. The androconia are involved in the production and dissemination of pheromones during courtship. These structures are greatly reduced in the *comet* mutant. Although such extreme pleiotropy appears to be the exception (it is not found in any other of our eyespot mutants), it does indicate the potential for effects across many wing characters.

C. Uncoupling of Eyespots

The phenotypic effects of several (but not all) mutants vary among the eyespots. *Spotty* adds eyespots to some wing cells but not to others. The localized effect on a single major vein in *cyclops* produces a more profound effect on the flanking eyespots than on others. Most strikingly, *A⁻* only affects the anterior of the two forewing eyespots. Most recently, butterflies have appeared in a line selected for fast preadult development in which two of the hindwing ventral eyespots (numbers three and four from the top, costal border of the wing) are absent or highly reduced while the other eyespots are unchanged. Early breeding results suggest a simple genetic basis for this pattern change. Intriguingly, one small subgroup of *Bicyclus* shows a very similar change in eyespot pattern (A. F. Monteiro, personal communication). We predict that these types of "uncoupling" genes with major phenotypic effects which are distributed in a nonuniform way across the eyespots have been extremely important in the context of evolutionary novelties and in promoting discrete patterns of morphological diversity. Moreover, the underlying developmental organisation of the eyespot character may generate bias such that certain combinations of eyespots can be readily uncoupled while other cannot (for example, perhaps while eyespots 3 and 4 can "drop out," combinations such as 1 and 2, or 4 and 5 cannot; see Keys *et al.*, 1999).

The effects of uncoupling leading to some degree of independence in development can be dramatic. One specific example is the extreme phenotypic plasticity of the ventral surface eyespots in contrast to an absence of plasticity in those on the dorsal wing surfaces of *B. anynana* and many other satyrine butterflies (Brakefield and Larsen, 1984; Brakefield and French, 1999). The

plasticity is induced by temperature and development time during the late larval period (Kooi and Brakefield, 1999). Two seasonal forms are found in nature, one in the wet season with large ventral eyespots and the other in the dry season with small eyespots. This seasonal polyphenism represents an adaptive response to differences in resting background and predator pressure between the seasons (Brakefield and Reitsma, 1991; Brakefield and French, 1999). The plasticity is mediated in the early pupae by the titers of ecdysteroid hormones (Koch *et al.*, 1996). The ventral eyespot foci of the seasonal forms differ in their signaling activity in early pupae (Brakefield *et al.*, 1996). We have recently used results from measurements of selected lines for eyespot size on either dorsal or ventral wing surfaces to suggest that one possible mechanism for this uncoupling between the wing surfaces is the presence of ecdysteroid receptors in cells of the ventral eyespot foci, but not in those of the dorsal wing surface (Brakefield *et al.*, 1998). Whatever the precise mechanism, this example demonstrates that novel means of regulating development of part of the eyespot pattern can be coopted during evolution. In fact, divergence between dorsal and ventral wing surfaces is an almost ubiquitous phenomenon of butterfly wing patterns. This is likely to be driven by butterfly behavior and natural selection but clearly mechanisms have evolved to facilitate independence in development at this level (see also Weatherby *et al.*, 1999).

DISCUSSION

We can now discuss again the most appropriate way of applying the term character to the eyespots of a butterfly such as *B. anynana*. There are several possible choices for the unit character: a single feature of an eyespot, a single eyespot, a subset of eyespots (e.g., those on one wing surface or dorsal/ventral), or the complete pattern. While arguments can be made for each of these from a morphological, genetical, or developmental viewpoint, the best overall choice is probably the complete pattern. Early descriptions of the morphology and variation across species indeed suggested that the unit character is most usefully taken as the whole pattern. This is now strongly supported by our understanding of the developmental mechanisms of eyespot formation from manipulative experiments and molecular research in *P. coenia* and *B. anynana*. All the empirical data are consistent with a single common developmental mechanism underlying the eyespots in these species and probably in all other Lepidoptera.

In our selection experiments on a specific eyespot feature there is a striking lack of substantial correlated responses for other features (cf. "black" and "gold" specimens in Fig. 4). While this has only been quantified for eyespot size and color, it probably holds for other pairs of features with the possible exception of additional "supernumerary" eyespots appearing in upward selected lines for eyespot size. The genetic correlations among eyespot features, even for the same individual eyespot, are thus low. For eyespot size and color, this is

presumably because whereas size is specified developmentally primarily by signal strength, the color composition is determined by threshold responses to the signal which correspond to boundaries between eyespot rings. This might suggest that from the perspective of genetics and development the different features should represent the unit characters rather than the complete eyespot pattern. However, it is difficult to imagine that natural selection frequently works in such a tightly targeted manner so that from an integrated evolutionary perspective, the choice of the eyespot pattern, inclusive of the various features, probably remains the most appropriate choice. This discussion also illustrates that more definitive descriptions of character structure, and also of evolutionary constraints, will require specific knowledge about mechanisms of natural selection on the eyespot pattern. We have performed some successful analyses of survival in cohorts of butterflies in the field to examine how visual selection by predators on butterflies at rest influences the size of the ventral eyespots in the seasonal forms (see Brakefield and French, 1999). However, such experiments will have to be greatly refined to produce the necessary detail about natural selection to match our understanding of character structure.

Condamin (1973) recognizes 77 species within the genus *Bicyclus*. These can have highly divergent eyespot patterns. There is also high species richness and diversity in many related genera (including *Mycalesis*). Many differences in the eyespot pattern, especially those which are quantitative and rather uniform across eyespots, are likely to reflect differentiation involving several or many eyespot patterning genes of small effect. There are indeed species of *Bicyclus* which correspond phenotypically to certain of the extreme phenotypes produced by our selection experiments in *B. anynana* (Brakefield, 1998). Such changes may be more characteristic of divergence among the most closely related species toward the tips of the phylogeny.

On the other hand, where more novel phenotypes are concerned, we believe that further study will eventually detect many examples where single genes have played a crucial role in the evolution of morphological diversity and character divergence across taxa. Such genes will be involved in some abrupt changes in features of all the eyespots, as well as in uncoupling certain eyespots leading to independent phenotypic effects across eyespots. *Spotty* may represent a gene of this type (Brakefield *et al.*, 1996). We hope to detect further examples in *B. anynana*. The availability of such genes, and thus the bounds of the developmental repertoire, may have yielded bias in evolutionary trajectories. Perhaps the potential pool of mutants overlaps considerably across species, even rather distantly related ones. If so, then mutagenesis in *B. anynana* may reveal some of the long-term potential for the eyespot character. Given sufficient time and large enough population sizes, absolute constraints in terms of genes or developmental options may have little relevance to the evolution of morphological diversity (although see the discussion of eyespot shape earlier). However, bias in the standing variation within populations is also likely to be

critical to the adaptive responses of populations, especially those involving tracking of rapid climate change. If populations on this time horizon tend to follow paths of least resistance in character evolution rather than alternatives, this may also contribute profoundly to shaping long-term patterns of morphological diversity. Further artificial selection experiments are being used to examine these ideas about the relevance of bias introduced through the standing genetic variation within populations (see Brakefield, 1998).

In terms of spectacular visual diversity, the evolution of the wing color pattern of butterflies appears to have behaved in a remarkably unconstrained manner. Although high morphological diversity may suggest extreme freedom in character evolution, such flexibility may be superficial. When different characters are considered, for example, the eyespot pattern as against the pattern of medial bands, there may effectively be few limits to independent evolution in terms of both direction and extent of change (Nijhout, 1991; Paulsen and Nijhout, 1993; Paulsen, 1994, 1996). Such complete independence in genetical, developmental, and evolutionary perspectives is perhaps the best evidence for the existence of different characters. In contrast, if the eyespot pattern indeed reflects a single character in terms of its evolutionary origins, and its genetical and developmental architecture, then evolution may be shaped not only by natural selection but also by bias in the generation of phenotypic variability. Evolution has progressed both by changing the character as a whole and, where possible, by decomposing a single unit into one or more partially independent modules. The type of detailed study at different levels of biological organization which we have begun for the eyespot pattern of *B. anynana* will provide the most detailed understanding of how such a character evolves.

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CHARACTERS AND ENVIRONMENTS: THE CONCEPT OF REACTION NORMS

When most biologists speak of a given "character," they usually think they know what they mean. A character can be continuous or discrete. For example, the height of a plant or the number of vertebrae in a reptile. In either case, it is usually obvious how to measure it. Things are only slightly more

tricky though the nature of morphological data is frequently inadequately represented by such terms as "discrete" or "continuous" (Rushworth, 1991; Rohlf and Manton, 1997; Manton et al., 1998).