

Development and evolution on the wing

W. Owen McMillan, Antónia Monteiro and Durrell D. Kapan

Butterfly wing patterns are more than just visually stunning examples of the evolutionary process. They are also emerging as exceptional model systems with which to link the developmental and genetic processes that generate morphological variation with the ecological and evolutionary processes that mould variation in natural populations. Work over the past few years has proceeded simultaneously on key developmental and evolutionary aspects of patterns on butterfly wings. Important clues into molecular and developmental events occurring during wing development are now available that refine our understanding of pattern formation. In addition, recent behavioural, field and molecular phylogenetic work places butterfly wing pattern change in a solid ecological and evolutionary context. There are still large gaps in our understanding, but current research priorities are well laid out and experimental methodologies are in place to address them. The challenge is to synthesize diverse research strategies into a cohesive picture of morphological evolution.

Butterfly wing patterns are thin mosaics created by overlapping thousands of coloured scales. Each scale, which is a modified sensory bristle [1], contains a single colour pigment and has a characteristic morphology [2]. Scales are tiled in rows laid down within a well-defined network of wing veins. In its simplest conception, pattern formation begins when groups of scales are specified to produce particular colour pigments. It is the size, shape and position of clusters of similarly coloured scales that produce the bewildering array of butterfly wing patterns. However, there is nothing particularly simple about these patterns. The developmental genetic programmes that specify the fate of wing scales are modified by natural and sexual selection to produce complex mosaics that can warn predators or blend perfectly with the environment.

Nearly all of the 12 000 described butterfly species can be distinguished by differences in wing pattern [2]. In spite of this daunting diversity, we continue to unravel the genetic, developmental and evolutionary underpinnings of butterfly wing patterns. Quantitative genetic and molecular developmental research is fuelling new ideas about how patterns form on butterfly wings. Insights into the molecular developmental details of pattern formation are being grounded in research that demonstrates the importance of butterfly wing patterns in adaptation [3,4], mate choice [5] and speciation [5,6]. Although there are still wide gaps in our understanding, there is perhaps no better animal system where the 'transitions from genes, through developmental pathways, to phenotype, function, and fitness' can be clearly illuminated [7]. The current challenge is to synthesize and expand this research to produce a cohesive picture of the interplay between development and evolution of butterfly wings.

Nature's palette

Research on butterfly wing pattern spans nearly 150 years and figures prominently in the maturation of developmental and evolutionary theory [2]. There are several reasons why butterfly wing patterns are such rich study systems. The patterns are essentially 2D structures, greatly simplifying the conceptualization and modelling of pattern change [2]. Additionally, butterfly wings are large and easy to manipulate. Haemolymph can be extracted from individual insects, and developing wing tissue can be excised and transplanted, revealing the developmental processes that give rise to these patterns (reviewed in [2,7,8]). Research on butterfly wing patterns also benefits from developmental genetic work on *Drosophila*. In spite of 230–280 million years of separation [9], the developmental 'toolkit' (*sensu* [10]) is remarkably preserved between flies and butterflies, and homologues of many of the patterning genes first identified in *Drosophila* have been identified in butterflies [10–15]. Lastly, and perhaps most importantly, wing patterns are the most prominent feature of a butterfly. Colour patterns have been strongly shaped by natural and sexual selection and often the agent of selection, whether predation, thermoregulation, mate choice, or a combination of these factors, can be readily identified and studied [4–6,12,16].

From pattern to process

Schwanwitsch and Süffert [17,18] recognized early last century that butterfly wing patterns are not just unique arrangements of pigmentation but rather that many different species share relatively few PATTERN ELEMENTS (see Glossary) [2]. They independently proposed a diagrammatic system of wing pattern homologies called the NYMPHALID GROUND PLAN (NGP) that was later expanded by Nijhout [2]. The NGP comprises a sequence of bands, eyespots and chevrons belonging to distinct SYMMETRY SYSTEMS that are repeated in each WING CELL (Box 1). It is important to understand, however, that the NGP is not a statement about how pattern elements are actually produced or modified during development. Rather it is a framework to help identify homologies between pattern elements across different species of Lepidoptera. Whether pattern elements hypothesized to be homologous under the NGP are actually homologous has been tested for only a few pattern elements in a very few species (see below and Box 2). Furthermore, the wing patterns of many species, particularly the bold pattern elements of

W. Owen McMillan*
Durrell D. Kapan
Dept of Biology,
University of Puerto Rico,
PO Box 23360, San Juan,
Puerto Rico.
*e-mail: wmcmillan@
rrpac.upr.clu.edu

Antónia Monteiro
Dept of Biological
Sciences, State University
of New York, Buffalo,
NY 14221, USA.

Box 1. The nymphalid ground plan and butterfly eyespots

The nymphalid ground plan (NGP) is an archetypal idealization of bands, eyespots, and chevron-shaped pattern elements (Fig. 1a). Under the NGP, butterfly wing patterns comprise a series of symmetry systems that includes the basal (purple) and central (dark blue) symmetry systems, border ocelli (yellow), parafocal elements (light blue), and submarginal and marginal bands (turquoise). Within this framework, the wing cell is the fundamental unit of pattern formation and each cell contains one or more elements of each symmetry system. Under the NGP hypothesis, the myriad of distinctive butterfly wing patterns is produced by the selective expression and/or distortion of these pattern elements. Experimental work on the NGP has been restricted to the Buckeye butterfly *Precis coenia* and the Forest Brown butterfly *Bicyclus anynana*, both of which bear eyespots and transversal bands. Work on these species has shown that there is much developmental and evolutionary flexibility in pattern production

among the different symmetry systems, among pattern elements within a symmetry system, and among components of the same pattern element. This flexibility provides astonishing raw material on which natural and sexual selection can act.

The eyespots of the border ocelli symmetry system are perhaps the simplest and mechanistically best understood of all butterfly pattern elements (Fig. 1b). The pigments of each eyespot are deposited in precise spatial relation around a central point or eyespot 'focus', often midway between the wing veins [a,b]. During the first hours following pupation, the focus signals to surrounding cells, which interpret the signal and subsequently differentiate into rings of differently coloured scales. In *Bicyclus*, artificial selection experiments and single gene mutants have revealed the underlying nature of variation in eyespot size, colour composition and shape [c–e]. Experimental ablation and focal-tissue transplants between each pair of selected lines indicate that each selection regime affects very different components of eyespot development. Artificial selection on a single eyespot in *B. anynana* gradually changes eyespot size (Low and High) by changing the eyespot signal and, to a lesser extent, the epidermal response to the eyespot signal. Colour composition lines (Gold and Black) vary in their response to the eyespot signal, whereas selection for eyespot shape (Fat and Thin) is accomplished partly by changes in the local epidermal cell arrangements. Diagrams below each eyespot represent a single wing cell.

Single gene mutants in *B. anynana* appear spontaneously in lab stock (Fig. 1c) and can cause large pattern changes in overall eyespot size (*Bigeye*), in colour composition (*Goldeneye* [f]), in eyespot shape (*Cyclops*, which simultaneously also varies in eyespot number and venation pattern), and in eyespot number – removing (3+4) or adding pairs of eyespots (*Spotty*) in a subset of the wing cells with no effect on the other pattern elements (reviewed in [g,h]).

References

- Nijhout, H.F. (1991) *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press
- French, V. and Brakefield, P.M. (1995) Eyespot development on butterfly wings: the focal signal. *Dev. Biol.* 116, 112–123
- Monteiro, A. *et al.* (1997) Butterfly eyespots: the genetics and development of the color rings. *Evolution* 51, 1207–1216
- Monteiro, A. *et al.* (1997) The genetics and development of an eyespot pattern in the butterfly *Bicyclus anynana*: response to selection for eyespot shape. *Genetics* 146, 287–294
- Monteiro, A.F. *et al.* (1994) The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. *Evolution* 48, 1147–1157
- Brunetti, C. R. *et al.* (2001) The generation and diversification of butterfly colour patterns. *Curr. Biol.* 11, 1578–1585
- Brakefield, P.M. (1998) The evolution-development interface and advances with the eyespot patterns of *Bicyclus* butterflies. *Heredity* 80, 265–272
- Brakefield, P.M. and French, V. (1999) Butterfly wings: the evolution of development of colour patterns. *BioEssays* 21, 391–401

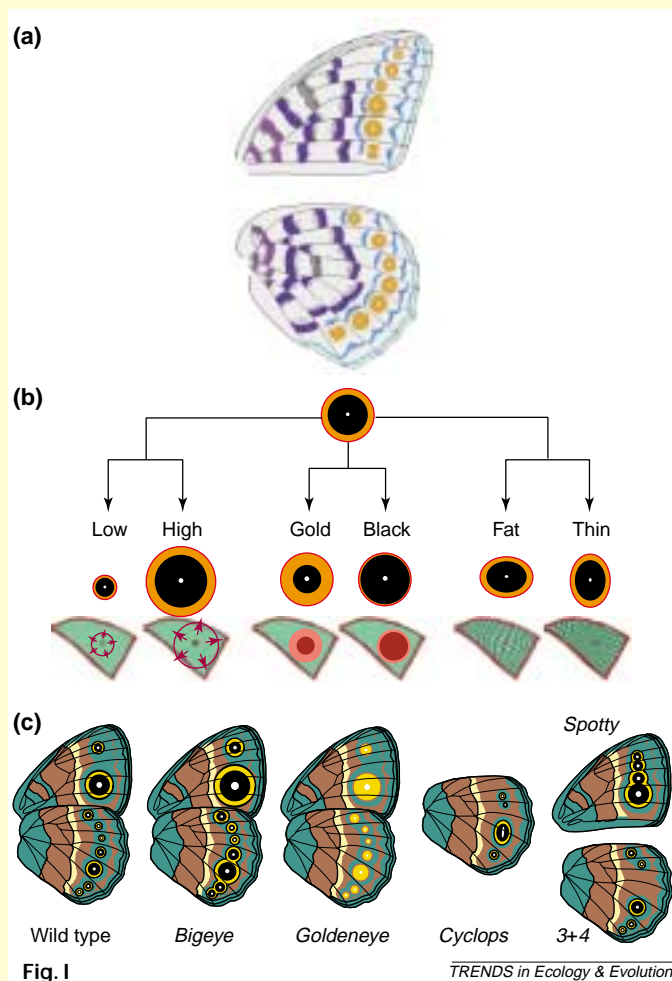


Fig. 1

TRENDS in Ecology & Evolution

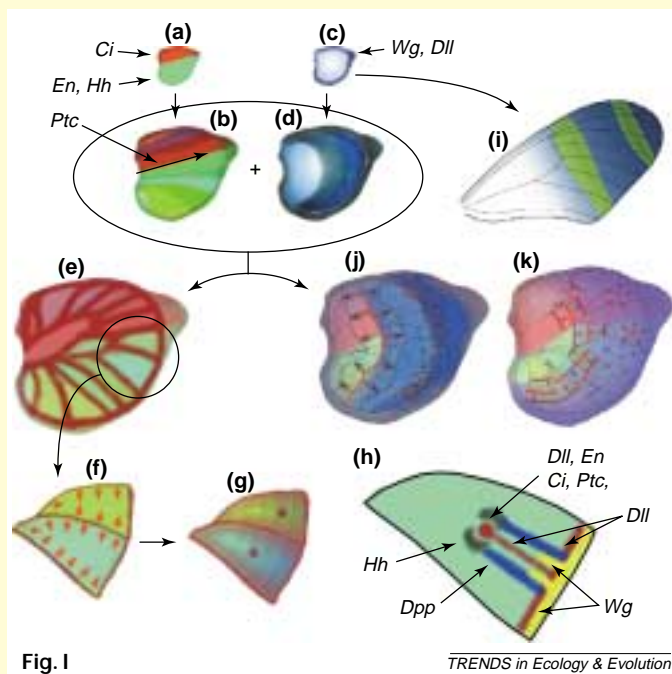
swallowtails *Papilio* (Box 3) and long wings *Heliconius* (Box 4), deviate substantially from the archetypal NGP. Fitting pattern change within these groups requires an extreme modification of the NGP and strongly suggests that novel patterning elements are involved [19] or that pattern formation in these species supersedes the NGP elements [20] (see below and Box 3).

Recent quantitative genetic research on pattern elements in the Buckeye butterfly *Precis coenia* focuses directly on the underlying developmental architecture of pattern evolution [21–24]. Different symmetry systems identified by the NGP appear to be genetically (and hence developmentally) autonomous and, therefore, able to evolve independently [21–24]. By contrast,

Box 2. A synthetic developmental genetic model of pre patterning in butterfly wings

Butterfly wing patterns might be set up by the redeployment of regulatory genes involved in vein patterning and overall wing growth, now hardwired through *cis*-regulatory evolution to pigment production pathways. Our synthesis incorporates recent work on *Drosophila* vein patterning [a]; homologues of *Drosophila* genes already identified in butterflies are indicated in Fig. 1 (Abbreviations: *Ci*, cubitus interruptus; *Dll*, distal-less; *Dpp*, decapentaplegic; *En*, engrailed or invected; *Hh*, hedgehog; *Ptc*, patched; *Wg*, wingless).

The anterior–posterior (AP) boundary (established very early in embryogenesis, Fig. 1a) is thought to be the initial source of patterning signals that, through several steps, subdivide the butterfly wing at the wing disc stage into separate genetic compartments along the AP axis (Fig. 1b). Simultaneously (Fig. 1c), long-range patterning morphogens, such as *wingless* (*Wg*) might be important in subdividing the wing into separate genetic compartments along the proximal–distal (PD) axis (Fig. 1d). Together, these two types of regional regulatory gene might be involved in positioning the longitudinal and cross veins in the butterfly wing (as they do in *Drosophila* [a]), which differentiate at boundaries of gene expression.



Vein markers (Fig. 1e), such as the putative butterfly homologue to the *Drosophila* gene, *rhomboid* (red), might become expressed at boundaries of gene expression, and define the future position of the veins. In turn, several other genes might be turned on in and around the 'future' vein tissue (Fig. 1h). These regulatory cascades, which might involve signalling from the presumptive veins and wing margin, and lateral inhibition processes, are repeated in each wing cell. In eyespot-bearing species, a central group of cells differentiates in the late larval wing disc (Fig. 1g), which, during the pupal stage, will be involved in eyespot signalling.

Complex gene regulatory cascades are probably involved in patterning each wing cell and in specifying eyespot foci (Fig. 1f). Evidence for this is seen in the great diversity of regulatory gene expression patterns in late larval wing discs of the Buckeye butterfly *Precis coenia* and the Forest Brown butterfly, *Bicyclus anynana* [b,c] (note that this figure is only a rough schematic interpretation of the spatial location of all the gene products or mRNAs and carries no information on their relative temporal patterns of expression). We propose that this patterning mechanism is modified in each wing cell by the different genetic backgrounds that were important in setting up the position of the veins (represented by the different shades of red and green in Fig. 1e). The interaction among the genes in Fig. 1h and the genes in Fig. 1e leads to the evolution and 'uncoupling' of pattern within a row of serially homologous elements such as the eyespots.

The bands of the central and basal symmetry systems might be determined by: prepattern genes responding in a threshold-like fashion to gradients established from the wing margin; boundaries of gene expression established by genes patterning the PD axis (Fig. 1d) and defining a row of cells that later become signalling centres for the bands (Fig. 1j); or genes patterning the AP axis (Fig. 1b) that interact with the PD axis-patterning genes (Fig. 1c or Fig. 1d), and make pieces of the band 'foci' dislocate when crossing a longitudinal vein boundary (Fig. 1k). There is experimental evidence for signalling from the eyespot organizers (as shown by the arrows in Fig. 1j and Fig. 1k) but no such evidence for signals emanating from putative band organizers (also shown with arrows).

References

- Biehs, B. *et al.* (1998) Boundaries in the *Drosophila* wing imaginal disc organize vein-specific genetic programs. *Development* 125, 4245–4257
- Carroll, S.B. *et al.* (1994) Pattern formation and eyespot determination in butterfly wings. *Science* 265, 109–114
- Keys, D.N. *et al.* (1999) Recruitment of a hedgehog regulatory circuit in butterfly eyespot evolution. *Science* 283, 532–534

elements belonging to the same symmetry system display less genetic independence, suggesting a common underlying developmental mechanism and concerted evolution [21–23]. Similarly, in the Forest Brown butterfly *Bicyclus anynana*, artificial selection on size, colour composition, or shape of a single eyespot produces correlated changes in other eyespots across the wing surface, but not in other features of the wing pattern [25–27] (reviewed in [7,8]). However, even pattern elements belonging to the same symmetry system can become developmentally 'uncoupled' and follow separate evolutionary trajectories. For example, in *P. coenia*, the genetic correlation between eyespots

with different morphologies is lower than that observed between morphologically similar eyespots [21–23]. This independence could be achieved by small gradual genetic changes or through major switches in developmental modularity (*sensu* [28]) that drastically affect pattern in only a subset of the wing cells (reviewed in [7,8], Box 1). In addition, different components of a single pattern element show a remarkable degree of developmental (and evolutionary) independence. For example, in *B. anynana*, selection for colour composition in one eyespot does not cause correlated change in the size of that eyespot. Likewise, selection for size produced only minor change in colour composition [25] (Box 1).

Box 3. The molecular basis of melanism in *Papilio glaucus*

Colour pattern polymorphism in the palatable Eastern tiger swallowtail *Papilio glaucus* is a classic example of Batesian or true mimicry. Females are either yellow-and-black striped (nonmimetic wild type) or mostly black (melanic) and mimic the distasteful Pipevine swallowtail *Battus philenor*. The switch from the nonmelanic to melanic form is controlled by a dominant allele at a Y-linked locus, plus a modifying X-linked locus that can suppress the melanic phenotype [a].

Wing colour pattern formation in *P. glaucus* is envisioned as a two-step process (Fig. 1a). The first step is colour prepatter determination during the late larval-early pupal stage by morphogen gradients emanating from the wing veins or margins (Box 2). This determines the colour pigments that make up the final pattern and which are not synthesized until late in the pupal stage, about two to three days before eclosion. In *P. glaucus*, pigment production is highly synchronized with the final stages of scale maturation (e.g. sclerotization), with the white scales maturing first (stage V), later becoming iridescent blue, followed by red and yellow (stage IV–III), and lastly black (stage II–I) [b,c].

In *Papilio*, biochemical pathways responsible for the final sclerotization of the scales and production of yellow-red (papiliochrome) and black (melanin) pigments are tightly coupled (Fig. 1b). Sclerotization is achieved primarily by cross-linking *N*-acetyl-dopamine (NADA) and *N*- β -alanyl-dopamine (N β AD), both derived directly from dopamine (Dopa), a precursor of both papiliochrome and melanin synthesis. Furthermore, *Papilio* seems to have co-opted N β AD from its role in sclerotization into

the synthesis of the yellow and red papiliochromes, which can also be synthesized from tryptophan via kynurenine [d].

In *P. glaucus*, darkening of the central forewing area is caused by a replacement of yellow papiliochromes with black melanin pigments, rather than by a mixture of black and yellow pigments. The shift from wild-type to melanic form is coincident with dynamic changes in regulation of two key enzymes, dopa decarboxylase (DDC) and *N*- β -alanyl-dopamine-synthase (BAS), which are involved in pigment synthesis and scale sclerotization [b–f]. DDC contributes dopamine both to early papiliochrome synthesis and to later melanin synthesis, and BAS shunts dopamine into the papiliochrome pathway (Fig. 1bi). In wild-type females, *Ddc* and *Bas* are both expressed early (stage IV) in the central forewing, where yellow pigments are ultimately laid down. By contrast, both enzymes are suppressed during stage IV in melanic females (Fig. 1bii). This delays maturation and leads to abnormal melanization during the final stages of pigmentation. Figure modified with permission from Richard ffrench-Constant.

References

- a Scriber, M. *et al.* (1996) Genetics of mimicry in the tiger swallowtail butterflies, *Papilio glaucus* and *P. canadensis* (Lepidoptera: Papilionidae). *Evolution* 50, 222–236
- b Koch, P.B. *et al.* (1998) Regulation of dopa decarboxylase expression during colour pattern formation in wild-type and melanic tiger swallowtail butterflies. *Development* 125, 2303–2313
- c ffrench-Constant, R. and Koch, P.B. Mimicry and melanism in swallowtail butterflies: towards a molecular understanding. In *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (Boggs, C.L. *et al.*, eds), University of Chicago Press (in press)
- d Koch, P.B. *et al.* (2000) The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr. Biol.* 10, 591–594

Developmental genetics on the wing

Models of pattern formation

Developmental models describe pattern formation as a two- [29] or three-step [2] process. First, a genetic PREPATTERN is laid down by two or more MORPHOGENS that diffuse from the wing margin or wing veins and that react with each other through LATERAL INHIBITION. This leads to the establishment of large (in whole-wing simulations [29]) or small (in wing-cell simulations [2]) areas with a pattern of stable morphogen concentration. In the three-step model [2], the wing surface prepatter is further refined when groups of differentiated cells become additional SOURCES (or SINKS) of pattern induction substances [2]. Finally, individual cells interpret the positional information laid down in the first two steps and respond by producing colour pigments. These models, although elegant, attack the problem of pattern formation on butterfly wings from a somewhat intermediate stage of wing development. They do not address either how particular areas of the wing margin become differentiated as sources of diffusible substances [29], or how pattern can be modulated independently in each wing cell [2].

Candidate genes for pattern formation

Genetic dissection of butterfly WING DISCS provides the first indication of the molecular events that might underlie early pattern formation on butterfly wings. Carroll and co-workers [11] showed that the molecular

developmental mechanisms of wing formation are similar between *Drosophila* and butterflies.

Additionally, they demonstrated that a *Precis* homologue of *distal-less*, a *Drosophila* REGULATORY GENE, is also expressed in the cells fated to be the 'future' eyespot centre in both *P. coenia* and *B. anynana* [11,12].

Homologues of many conserved regulatory genes, and even whole regulatory pathways, are co-opted to play a role in colour pattern formation (reviewed in [30], Box 2). For example, *wingless* and *decapentaplegic*, which are long-range diffusible morphogens [31–33], are expressed in the forewing of *Precis* in areas that correspond to the future bands of the CENTRAL SYMMETRY SYSTEM, and in pairs of short marginal rays within the wing cell, respectively [11]. Furthermore, several genes from the *hedgehog* signalling pathway are deployed in and around the eyespot foci in *Precis* suggesting that, just like *distal-less*, they might also play a role in eyespot development [34]. Given the growing number of genes with suggestive expression patterns, it is now crucial to test whether they do have a functional role in pattern formation.

Developmental genetics and pattern formation: a new synthesis

The research that we have discussed identifies important candidate patterning genes, but does not address how conserved regulatory cascades change across the different wing cells and across different

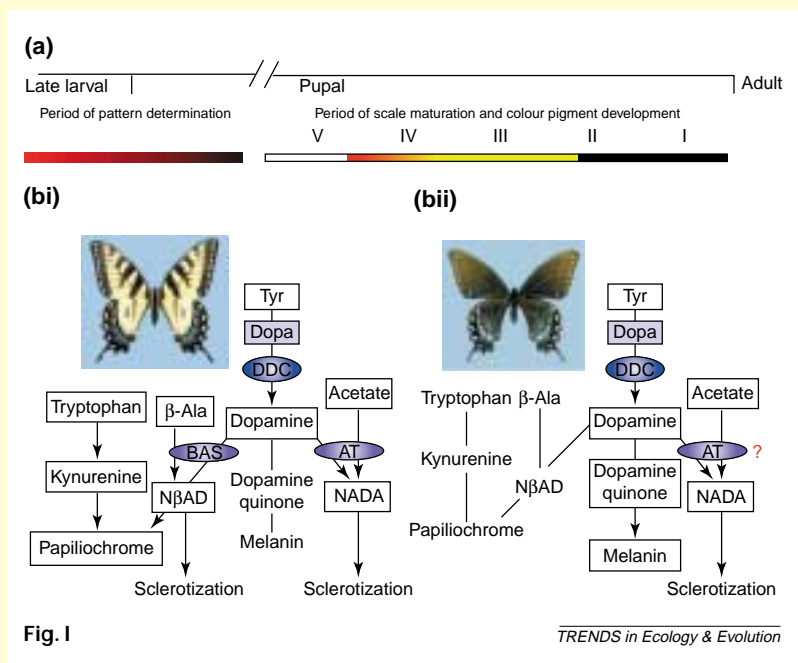


Fig. 1

TRENDS in Ecology & Evolution

e Koch, P.B. *et al.* (2000) Butterfly wing pattern mutants: developmental heterochrony and co-ordinately regulated phenotypes. *Dev. Genes Evol.* 210, 536–544

f Koch, P.B. *et al.* (2000) Insect pigmentation: activities of Beta-alanyldopamine syntase in wing color patterns of wild-type and melanic mutant swallowtail butterfly *Papilio glaucus*. *Pig. Cell Res.* 13, 54–58

species. A synthesis of recent empirical and theoretical research on butterfly wing-patterns that incorporates elements of both wing and wing vein development in *Drosophila* is presented in Box 2. Under our model, pattern formation is the result of several genetic prepatterning mechanisms that are coupled at different stages in development to pigment and scale maturation pathways (Box 3). Tissue differentiation usually accompanies tissue growth – a small wing pouch grows as it becomes subdivided into a larger number of genetic compartments. This genetic subdivision of the wing can be accomplished by the propagation of cells and cell lines that are fated, very early on, to express certain transcription factors. Additionally, the diffusion of morphogens produced at boundaries between the previously fated cell types can further differentiate populations of cells in the wing [35]. Because morphogen diffusion in a cellular environment is thought to be limited in range, the broader pattern elements, such as the bands of the central symmetry system or the transversal wing band of many *Heliconius* spp. (Box 4), might be specified by genetic prepatterning laid out when the developing wing disc is still relatively small and before the position of wing veins has been specified [20]. The finer details of the pattern, especially those that are repeated within each wing cell, might derive from developmental processes that take place at the same time or after the position of the wing veins is established.

During vein development in *Drosophila*, longitudinal veins form at boundaries of expression of regional regulatory genes [36]. The domains of expression of these genes are determined by signalling mechanisms derived from the anterior–posterior (AP) boundary, which is set up very early in wing development. It is likely that similar vein-determining mechanisms are preserved in butterflies. If so, this genetic subdivision of the wing, which changes composition at vein boundaries can, in principle, also function to modify individual pattern elements within a symmetry system (e.g. by 'dislocating' pieces of the bands as they cross a wing vein, or by giving each eyespot its own characteristic morphology). A similar function might apply to genes patterning the proximal–distal (PD) axis of the wing. In this case, these genes could be involved in shifting the position of the eyespots or bands along the PD axis of the wing. Major and minor changes to the pattern elements in subsets of the wing cells might involve *CIS-REGULATORY* mutations affecting upstream regulators or the downstream targets of these genes.

Scale maturation and pigmentation

Ultimately, patterns on butterfly wings are generated when scale-producing cells interpret prepatterning information and respond by developing specific scale morphologies and producing specific colour pigments. These final steps of pattern formation occur late in wing development [2] (Box 3). Recent research by Koch and co-workers on melanization in the Eastern tiger swallowtail *Papilio glaucus* helps untangle the links between early and late stages of pattern development by providing insight into (1) the types of molecules that are responding to prepatterning information; (2) the spatial and temporal scales over which patterning signals are being interpreted; and (3) the interactions between the pigment biochemical cascades and scale cell maturation rates [37–40] (Box 3). Colouration is highly ordered in all butterfly species that have been examined, with coloured scales (whites, reds and yellows) developing before black (melanized) scales [37,40,41]. In *P. glaucus*, at least, pattern change is achieved by finely coordinating the expression of two genes causing concordant changes in both pigment synthesis and scale maturation [37–39] (Box 3).

Koch *et al.* [37–39] nicely demonstrated how simple biochemical switches have profound phenotypic effects on colouring the existing genetic prepatterning. The observation that colouration is ordered and highly synchronized with scale maturation led them to propose that pattern change in butterflies is modulated by heterochronic changes in scale development. Under their model, varying the rate of scale maturation can change the final colour, position and/or shape of wing pattern elements [40]. Thus, one of the effects of the prepatterning genes might be to regulate the developmental rate of scales in different areas of the wing, but this conclusion awaits empirical demonstration.

Box 4. Ecology and evolution of wing patterns in *Heliconius*

The bold warning colours of *Heliconius* butterflies are the best-known example of Müllerian mimicry and there is striking variation in wing patterns at every level.

At a given locality in the Neotropics, butterfly colour-pattern diversity converges to three to five different mimicry rings of heliconiines and other aposematic species [a]. For example, Fig. 1a illustrates members of four different sympatric mimicry rings from west Costa Rica. Within each ring the wing patterns of heliconiine (*H. erato* and *H. hewitsoni* Fig. 1ai, ii) and ithomiine (*Tithorea* and *Melinaea* Fig. 1aiii, iv) co-models, are matched by *Heliconius* spp. from the rapidly evolving melpomene–cydno–sylvaniform (MCS) clade (Fig. 1av–viii).

In addition, the wing patterns of species within mimicry rings often change as one moves across Central and South America (Fig. 1b). This pattern of divergence is most noticeable in *H. erato* (Fig. 1bi) and *H. melpomene* (Fig. 1bii), two important species of the 'red' mimicry ring. At any given locality, these two species have identical patterns, yet there has been concordant diversification to produce ~25 colour pattern races. Shown here are four pairs of *H. erato* and *H. melpomene* races from Costa Rica, Ecuador and Peru. Interestingly, molecular data suggest that the concordant changes in wing patterns that define the two co-models are not produced by strict co-evolutionary change [b].

Colour pattern variation extends to local populations of the same 'geographical' race (Fig. 1c). Both *H. numata* and *H. cydno*, closely related species in the MCS clade, can be polymorphic. In

Fig. 1c are six morphs of *H. numata*, each belonging to the 'tiger' mimicry ring, collected in a 25-km² area of forest in Peru [c].

Although very little is known about pattern development in *Heliconius*, the genetic architecture of pattern change is well described. As in *Papilio* (Box 3), scale type and colour are correlated [d] and major 'switch' genes, which initiate particular developmental cascades, control the colour, position and shape of the pattern elements [e,f]. Polymorphism within a given geographical region is caused by allelic substitutions at one or two loci in *H. numata* and *H. cydno*, respectively (Fig. 1c) [c,g]. The remarkable colour-pattern radiation that characterizes *H. erato* and *H. melpomene* is more complex, but can largely be explained by allelic changes at five or six unlinked loci (Fig. 1b) [e,f].

The genetic 'toolbox' for creating novel wing patterns is preserved across species boundaries [h], even when speciation is associated with a radical mimetic switch [h,i]. Thus, colour-pattern differences between closely related species are caused by the same loci that are responsible for phenotypic differences within a species. However, it is unclear how far across the phylogeny this conclusion extends. It is unknown whether the convergent wing patterns of co-models *H. erato* and *H. melpomene* (Fig. 1b), are the result of change at homologous pattern loci or are brought about by the action of different loci. None of the patterning genes have phenotypic effects that are completely identical in the two species. Moreover, the same pattern element (e.g. the forewing band colour) appears to be under different genetic control [i,j].

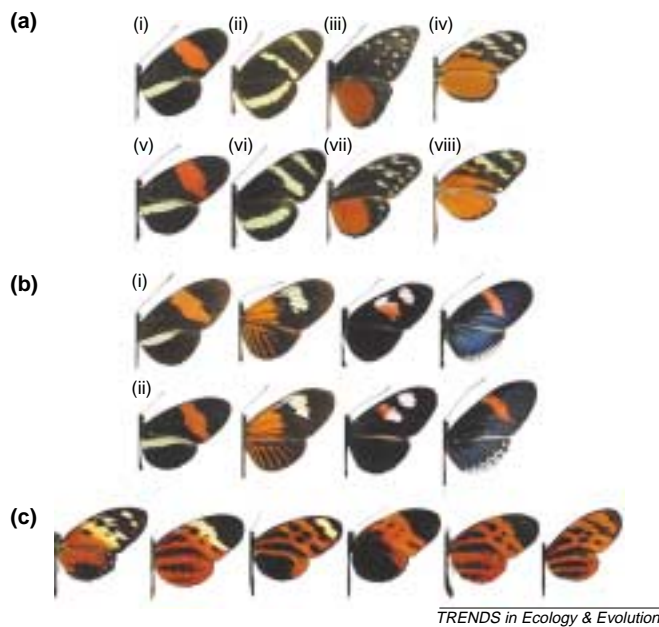


Fig. 1

References

- a Joron, M. and Mallet, J. (1998) Diversity in mimicry: paradox or paradigm? *Trends Ecol. Evol.* 13, 461–466
- b Brower, A.V.Z. (1996) Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution* 50, 195–221
- c Joron, M. *et al.* (2001) Variable selection and the coexistence of multiple mimetic forms in *Heliconius numata*. *Evol. Ecol.* 13, 721–754
- d Gilbert, L.E. *et al.* (1988) Correlations of ultrastructural and pigmentation suggest how genes control development of wing scales on *Heliconius* butterflies. *J. Res. Lepidopt.* 26, 141–160
- e Nijhout, H.F. (1994) Developmental perspectives on the evolution of butterfly mimicry. *BioScience* 44, 148–157
- f Nijhout, H.F. (1991) *The Development and Evolution of Butterfly Wing Patterns*, Smithsonian Institution Press
- g Kapan, D.D. (2001) Three-butterfly system provides a field test of Müllerian mimicry. *Nature* 409, 338–340
- h Jiggins, C.D. and McMillan, W.O. (1997) The genetic basis of an adaptive radiation: warning colour in two *Heliconius* species. *Proc. R. Soc. London B Biol. Sci.* 246, 1167–1175
- i Gilbert, L.E. Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for shared genetic 'toolbox' from synthetic hybrid zones and a theory of diversification. In *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (Boggs, C.L. *et al.*, eds), University of Chicago Press (in press)
- j Mallet, J. (1989) The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proc. R. Soc. London B Biol. Sci.* 236, 163–185

The ecological and evolutionary significance of butterfly wing patterns

Genetic changes affecting pigment pathways and their developmental control generate variation in adult butterfly wing patterns (Boxes 1–3). However, ecological and evolutionary forces shape this variation and, to an extent not yet fully understood, determine

the kinds of developmental modifications that become fixed and underlie species differences. The patterns on butterfly wings are attractive model systems because the selective forces that complete the integrative feedback loop between genes, development, form and function are often strong, identifiable and variable both within and across species.

Box 5. Questions for future research

- What are the connections between early pattern signals and the biochemical switches that colour the wing during the final stages of pattern development? We have a rudimentary understanding of early eyespot gene expression and biochemical events in late pigment synthesis. Further research is required to understand the interactions between these two processes.
- Are loci implicated by gene expression studies directly involved in pattern formation and probable targets for evolutionary change? Suggestive expression patterns do not prove causation, and future work should determine whether genes expressed within future colour pattern elements actually play a causal role in pattern formation.
- How general are the lessons that we have learned about eyespot pattern formation? Developmental research on eyespots captures very little of the complexity in butterfly wing patterns. Additional developmental research on processes underlying pattern formation in ecologically and evolutionary well-studied groups, such as *Heliconius* and *Papilio*, is required.
- How do evolutionary and ecological pressures shape the genetic and developmental architecture of colour pattern formation? Genetic changes underlying eyespot variation in *Bicyclus* differ from those regulating melanism in *Papilio* and warning colour divergence in *Heliconius* (Boxes 1,3,4). Full appreciation of pattern evolution requires an understanding of how internal developmental processes have been shaped by diverse selective pressures.

Butterfly wing patterns continue to provide excellent case studies of adaptation and adaptive change. Many species, including *Precis* and *Bicyclus*, show phenotypic plasticity, or SEASONAL POLYPHENISM, in wing patterns. The different seasonal wing pattern forms are adapted to changing environmental conditions and highlight the fine integration between natural selection and developmental programmes that control pattern formation. Research on seasonal polyphenisms, including the field estimates of selection and genetic and hormonal control of pattern change, has recently been reviewed [3,8,42]. Recent molecular phylogenetic work is expanding our understanding polyphenism and pattern change in *Bicyclus* spp. [43], which differ in their degree of polyphenism [44] and display a diverse array of wing patterns that consist mostly of variation in eyespot number, size, position and colour composition, as well as variation in the banding pattern on the ventral and dorsal wing surfaces. The new molecular phylogeny of the group provides an essential framework to map this variation and determine which changes are preferred by selection [43] (Box 2).

The evolutionary significance of wing patterns is perhaps best understood in the Neotropical *Heliconius*, a group characterized by striking diversity in wing colouration (reviewed in [20], Box 4). At the most basic level, *Heliconius* wing patterns are adaptations that warn potential predators of their unpalatability. The selective benefit that one *Heliconius* species accrues through Müllerian mimicry with another *Heliconius* species has recently been demonstrated [4]. Importantly, the strength of natural selection against nonmimetic wing patterns varies inversely with the density of butterflies released. Thus, only relatively few novel patterned distasteful butterflies can effectively 'educate' potential bird predators within an area, providing one of the first demonstrations that selection is relaxed when nonmimetic forms are released at high density, as was theorized recently [45]. The finding that the adaptive landscape of wing patterns is sensitive to changes in density helps explain the existence of sympatric polymorphism

within Müllerian mimics and provides new insights into the rapid origin and radiation of new mimetic patterns [4] (reviewed in [45,46], see Box 4).

The vivid wing patterns of *Heliconius* are also important in speciation. For example, speciation between *H. himera* and *H. erato* appears to have been catalysed by the association of strong mating preference with divergence in warning colouration [47]. Colour-pattern change might also cause speciation by directly influencing mate choice and/or species recognition. New research on *H. melpomene* and its sister species *H. cydno* demonstrates that the evolution of divergent mimetic wing colour patterns causes changes in mating preference [5]. This pleiotropic effect could directly cause speciation or it could combine with other factors, such as microhabitat segregation or hybrid dysfunction, to precipitate the formation of new species [5].

Phylogenetic work on *Heliconius* provides an important backdrop to explore pattern evolution. The genus can be broadly divided into two major clades, the erato–sara–sapho clade (ESS) and the melpomene–cydno–sylvaniform clade (MCS), which diverged from each other early in the evolutionary history of the group [48]. Each clade demonstrates a clear association between speciation and marked changes in wing colouration that result in shifts between MIMICRY RINGS (reviewed in [16], Box 4). This work also documents the extraordinary speed at which colour pattern evolution can proceed. The members of the MCS clade, which differ in mimetic alliance (Box 4), diverged from each other within the last two to four million years [48]. Intraspecific variation in wing pattern that characterizes the two CO-MODELS, *H. erato* and *H. melpomene*, appears to have evolved even more recently [46,49,50].

Conclusions and future research directions

Considerable progress has been made in our understanding of pattern formation on butterfly wings; however, wide gaps remain and a few of the outstanding questions are highlighted in Box 5. Our understanding of pattern formation will continue to

Acknowledgements

The authors thank Patricia Beldade, Shannon Bennett, Paul Brakefield, Vernon French, Richard French-Constant, Mathieu Joron, Jim Mallet, Fred Nijhout, Alexandra Tobler and two anonymous referees for comments; Fred Nijhout for letting us colourize his NGP figure; Larry Gilbert and Mathieu Joron for access to their extensive collections of *Heliconius*; Bernard Koch and Richard French-Constant for photos of *Papilio glaucus*. W.O.M. was supported by a National Science Foundation grant (DEB-9806792). A.M. was funded by a Human Frontiers Science Program grant (RG0058) and co-authored this article whilst at the Institute of Evolutionary and Ecological Sciences, University of Leiden, Leiden, The Netherlands. The authors contributed equally to this article.

Glossary

Central symmetry system: a central wing band comprising a broad homogeneous central area with an axis of symmetry about its centre (e.g. a white and a brown border on one side and a brown and a white border on the other side).

Co-models: two or more distasteful warningly coloured (aposematic) species that share similar or identical patterns.

Cis-regulatory region: a noncoding section of DNA that is positioned relatively close to the coding region of a gene and that is responsible for the activation or repression of that gene.

Lateral inhibition: two chemicals diffuse freely whilst reacting with each other. The activator, whose synthesis is subject to strong feedback (autocatalysis), diffuses at a slower rate than does the inhibitor, which suppresses autocatalysis around an area of activator production. Eventually, this system gives rise to dynamically stable patterns of activator and inhibitor.

Mimicry ring: a group of sympatric species that share a common aposematic pattern.

Morphogen: a secreted signal that elicits different responses at different concentrations. A morphogen is (1) synthesized in a subset of cells; (2) diffuses from its site of synthesis to become progressively less concentrated further from the source of synthesis; and (3) cells respond to different concentrations of it by activating expression of distinct sets of genes.

Morpholinos: a synthetic oligonucleotide (up to 25 bp in length) that inhibits translation of mRNA molecules with a complementary sequence and effectively knocks out gene expression.

Nymphalid ground plan: a system of pattern homologies that outlines the maximum number of pattern elements of a certain type that can exist in a butterfly.

Pattern elements: morphological units of colour pattern that share the same characteristics.

Prepattern: an 'invisible' pattern of genetically differentiated cells, expressing different combinations of regulatory genes, which later can be translated into distinctly pigmented areas by influencing different pigment production pathways.

Regulatory gene: a gene whose function is the regulation of other genes, either by enhancing or repressing their transcription or through binding to the *cis*-regulatory regions of target genes.

RNA interference: a method that employs *in vitro* engineered double-stranded RNA that impairs the synthesis of the protein encoded by the complementary mRNA sequence of the host and effectively knocks out gene expression.

Seasonal polyphenism: alternative phenotypes displayed by individuals with the same genotype, caused when environmental factors characteristic of a particular season alter pattern development.

Sinks: single differentiated cells, a group of cells, wing veins or wing margins that degrade molecules from nearby cells.

Sources: single differentiated cells, a group of cells, wing veins or margins that produce molecules responsible for pattern induction.

Symmetry system: pattern elements that share a similar morphology and that are either continuous across the wing (e.g. the central band) or repeated in each wing cell (e.g. the row of border ocelli), and that have a central axis of symmetry (with respect to their colouration) running through their middle and separating the pattern into a proximal and a distal half. Eyespots can often show complete radial symmetry.

Wing cell: the area of the wing bordered by wing veins.

Wing discs: wings growing inside the thoracic cavity of a larva.

grow as we learn more about regulatory systems from the genetic dissection of *Drosophila* and other model organisms. Molecular techniques, such as RNA INTERFERENCE and MORPHOLINOS, which allow a transient knockout of a target gene and the recent development of transgenic techniques in Lepidoptera [51], will help explain how gene expression changes translate into wing pattern change. Moreover, the increasing pace of developmental work on other groups, such as *Heliconius* and *Papilio*, will help to build a more comprehensive view of butterfly wing pattern formation. Genetic mapping studies are progressing in both *Heliconius* and *Papilio*. Thus,

in the future, genes found to be important in pattern formation in *Precis* and *Bicyclus* can be simultaneously followed in *Heliconius* and *Papilio*, and mapped relative to the genes known to underlie colour pattern change. As colour-pattern loci and their regulatory regions are located and sequenced, it will be possible to probe directly the history of the genetic substitutions that lead to adaptive change or that underlie species differences. This information, coupled with the identification of selection pressures acting on wing patterns, will allow a glimpse of one of the most fascinating and colourful interplays between genes, development and evolution.

References

- Galant, R. *et al.* (1998) Expression pattern of a butterfly *acheate-scute* homolog reveals the homology of butterfly wing scales and insect sensory bristles. *Curr. Biol.* 8, 807–813
- Nijhout, H.F. (1991) *The Development and Evolution of Butterfly Wing Patterns*, Smithsonian Institution Press
- Brakefield, P.M. (1996) Seasonal polyphenism in butterflies and natural selection. *Trends Ecol. Evol.* 11, 275–277
- Kapan, D.D. (2001) Three-butterfly system provides a field test of Müllerian mimicry. *Nature* 409, 338–340
- Jiggins, C.D. *et al.* (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* 411, 302–305
- McMillan, W.O. *et al.* (1997) What initiates speciation in passion-vine butterflies? *Proc. Natl. Acad. Sci. U. S. A.* 94, 8628–8633
- Brakefield, P.M. (1998) The evolution–development interface and advances with the eyespot patterns of *Bicyclus* butterflies. *Heredity* 80, 265–272
- Brakefield, P.M. and French, V. (1999) Butterfly wings: the evolution of development of colour patterns. *BioEssays* 21, 391–401
- Wootton, R.J. (1981) Palaeozoic insects. *Annu. Rev. Entomol.* 26, 319–344
- Carroll, S.B. *et al.* (2001) *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design*, Blackwell Scientific
- Carroll, S.B. *et al.* (1994) Pattern formation and eyespot determination in butterfly wings. *Science* 265, 109–114
- Brakefield, P.M. *et al.* (1996) Development, plasticity, and evolution of butterfly eyespot patterns. *Nature* 384, 236–242
- Weatherbee, S.D. *et al.* (1998) *Ultrabithorax* regulates genes at several levels of the wing-patterning hierarchy to shape the development of the *Drosophila* haltere. *Genes Dev.* 12, 1474–1482
- Weatherbee, S.D. *et al.* (1999) *Ultrabithorax* function in butterfly wings and the evolution of insect wing patterns. *Curr. Biol.* 9, 109–115
- Warren, R.W. *et al.* (1994) Evolution of homeotic gene regulation and function in flies and butterflies. *Nature* 372, 458–461
- Mallet, J. *et al.* (1998) Mimicry and warning color at the boundary between microevolution and macroevolution. In *Endless Forms: Species and Speciation* (Howard, D. and Berlocher, S., eds), pp. 390–403, Oxford University Press
- Schwannitsch, D.S. (1924) On the groundplan of wing-pattern in nymphalids and certain other families of rhopaloceros Lepidoptera. *Proc. Zool. Soc. London B* 34, 509–528
- Süffert, F. (1927) Zur vergleichende Analyse der Schmetterlingszeichnung. *Biol. Zent.* 47, 385–413
- Mallet, J. (1991) Variations on a theme? *Nature* 354, 368
- Gilbert, L.E. Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for shared genetic 'toolbox' from synthetic hybrid zones and a theory of diversification. In *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (Boggs, C.L. *et al.*, eds), University of Chicago Press (in press)
- Paulsen, S.M. and Nijhout, H.F. (1993) Phenotypic correlation structure among elements of the color pattern in *Precis coenia* (Lepidoptera: Nymphalidae). *Evolution* 47, 593–618
- Paulsen, S.M. (1994) Quantitative genetics of butterfly wing color patterns. *Dev. Genet.* 15, 79–91
- Paulsen, S.M. (1996) Quantitative genetics of the wing color pattern in the buckeye butterfly (*Precis coenia* and *Precis evarete*): evidence against the constancy of g. *Evolution* 50, 1585–1597
- Nijhout, H.F. (1994) Symmetry systems and compartments in lepidopteran wings: the evolution of a patterning mechanism. *Development*, (Suppl.), 225–233
- Monteiro, A. *et al.* (1997) Butterfly eyespots: The genetics and development of the color rings. *Evolution* 51, 1207–1216

- 26 Monteiro, A.F. *et al.* (1994) The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. *Evolution* 48, 1147–1157
- 27 Monteiro, A. *et al.* (1997) The genetics and development of an eyespot pattern in the butterfly *Bicyclus anynana*: response to selection for eyespot shape. *Genetics* 146, 287–294
- 28 Wagner, G. (1996) Homologues, natural kinds, and the evolution of modularity. *Am. Zool.* 36, 36–43
- 29 Sekimura, T. *et al.* (2000) A model for colour pattern formation in the butterfly *Papilio dardanus*. *Proc. R. Soc. London B Biol. Sci.* 267, 851–859
- 30 Carroll, S.B. (2000) Endless forms: the evolution of gene regulation and morphological diversity. *Cell* 101, 577–580
- 31 Entchev, E.V. *et al.* (2000) Gradient formation of the TGF-beta homolog *Dpp*. *Cell* 103, 981–991
- 32 Strigini, M. and Cohen, S.M. (2000) *Wingless* gradient formation in the *Drosophila* wing. *Curr. Biol.* 10, 293–300
- 33 Teleman, A.A. and Cohen, S.M. (2000) *Dpp* gradient formation in the *Drosophila* wing imaginal disc. *Cell* 103, 971–980
- 34 Keys, D.N. *et al.* (1999) Recruitment of a hedgehog regulatory circuit in butterfly eyespot evolution. *Science* 283, 532–534
- 35 Lawrence, P.A. (1992) *The Making of a Fly*, Blackwell Science
- 36 Biehs, B. *et al.* (1998) Boundaries in the *Drosophila* wing imaginal disc organize vein-specific genetic programs. *Development* 125, 4245–4257
- 37 Koch, P.B. *et al.* (2000) The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr. Biol.* 10, 591–594
- 38 Koch, P.B. *et al.* (2000) Insect pigmentation: activities of Beta-alanyl dopamine synthase in wing color patterns of wild-type and melanic mutant swallowtail butterfly *Papilio glaucus*. *Plg. Cell Res.* 13 (Suppl. 8), 54–58
- 39 Koch, P.B. *et al.* (1998) Regulation of dopa decarboxylase expression during colour pattern formation in wild-type and melanic tiger swallowtail butterflies. *Development* 125, 2303–2313
- 40 Koch, P.B. *et al.* (2000) Butterfly wing pattern mutants: developmental heterochrony and co-ordinately regulated phenotypes. *Dev. Genes Evol.* 210, 536–544
- 41 Gilbert, L.E. *et al.* (1988) Correlations of ultrastructural and pigmentation suggest how genes control development of wing scales on *Heliconius* butterflies. *J. Res. Lepidop.* 26, 141–160
- 42 Nijhout, H.F. (1999) Control mechanisms of polyphenic development in insects. *BioScience* 49, 181–192
- 43 Monteiro, A. and Pierce, N.E. (2001) Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from *COI*, *COII*, and *EF-1A* gene sequence. *Mol. Phylog. Evol.* 18, 264–281
- 44 Roskam, J.C. and Brakefield, P.M. (1996) A comparison of temperature-induced polyphenism in African *Bicyclus* butterflies from a savannah-rainforest ecotone. *Evolution* 50, 2360–2372
- 45 Joron, M. and Mallet, J. (1998) Diversity in mimicry: paradox or paradigm? *Trends Ecol. Evol.* 13, 461–466
- 46 Mallet, J. (1999 (2001)) Causes and consequences of a lack of coevolution in Müllerian mimicry. *Evol. Ecol.* 13, 777–806
- 47 Jiggins, C.D. and McMillan, W.O. (1997) The genetic basis of an adaptive radiation: warning colour in two *Heliconius* species. *Proc. R. Soc. London B Biol. Sci.* 246, 1167–1175
- 48 Brower, A.V.Z. and Egan, M.G. (1997) Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: Heliconiini): a revised phylogenetic position for *Eueides* based on sequences from mtDNA and a nuclear gene. *Proc. R. Soc. London B Biol. Sci.* 264, 969–977
- 49 Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. U. S. A.* 91, 6491–6495
- 50 Brower, A.V.Z. (1996) Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution* 50, 195–221
- 51 Tamura, T. *et al.* (2000) Germline transformation of the silkworm *Bombyx mori* L. using a piggyBac transposon-derived vector. *Nat. Biotechnol.* 18, 81–84

The honesty of bird song: multiple constraints for multiple traits

Diego Gil and Manfred Gahr

The function of bird song is closely linked to sexual selection. A fundamental question regarding the evolution of sexually selected male signals is how their honesty is maintained. The neural space required for storing a large song repertoire size has traditionally been identified as a key constraint. However, it is often forgotten that bird song is a multifaceted behaviour, and that the different characters that comprise it have specific costs. Recent research has revealed the existence of new constraints, such as social aggression or learning opportunities, which limit the expression of several song characteristics. We review the existing evidence for each of these constraints, revealing some major gaps in our knowledge of this fascinating biological system.

The two main functions of song in male birds are mate attraction and territory defence against other males [1]. Individual variation in song characteristics does affect reproductive success through mate choice and male–male competition, the two mechanisms of sexual selection [2]. Current theory predicts that when senders and receivers have different evolutionary interests, as in sexual selection, signals must be costly (i.e. subject to some constraint) to constitute stable, honest indicators of quality [3]. Individual variation in the expression of

these signals will therefore depend on the condition of the male [4]. Thus, differences in phenotypic or genetic quality between males would result in differences in song production. Evidence for condition dependency of song characteristics is, however, scant and often controversial [5]. A problematic feature of bird song is that it constitutes a set of characters rather than a simple trait, and each of these characters can be limited by specific constraints, a fact that is commonly ignored in the literature. Here, we consider which song characteristics are, or can be, sexually selected, and examine how different costs and constraints can limit the expression of each of these characteristics.

Bird song: multiple sexually selected traits

Bird song encompasses multiple traits that can be sexually selected, but which are not equally important in all species. We expect that characteristics with the greatest individual variation and repeatability will be those that are most important in the context of sexual selection.