Alternative models for the evolution of eyespots and of serial homology on lepidopteran wings

Antónia Monteiro

Summary
Serial homology is widespread in organismal design, but the origin and individuation of these repeated structures appears to differ with the different types of serial homologues, and remains an intriguing and exciting topic of research. Here I focus on the evolution of the serially repeated eyespots that decorate the margin of the wings of nymphalid butterflies. In this system, unresolved questions relate to the evolutionary steps that lead to the appearance of these serial homologues and how their separate identities evolved. I present and discuss two alternative hypotheses. The first proposes that eyespots first appeared as a row of undifferentiated repeated modules, one per wing compartment, that later become individuated. This individuation allowed eyespots to appear and disappear from specific wing compartments and also allowed eyespots to acquire different morphologies. The second hypothesis proposes that eyespots first appeared as individuated single units, or groups of units, that over evolutionary time were co-opted into new compartments on the wing. I discuss the merits of each of these alternate hypotheses by finding analogies to other systems and propose research avenues for addressing these issues in the future.
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Modularity of body plans and serially repeated structures is widespread in the animal kingdom.[1] Examples of modular structures include vertebrae,[2] teeth,[3] limbs,[4] digits,[5] arthropod body segments,[6] *C. elegans* terminal rays,[7] insect fore and hindwings[8–10] and butterfly eyespot patterns.[11–13] Some of the key questions driving research in the field of modularity seek to understand how such modules become repeated and also how they acquire the ability to differentiate into more or less distinct structures.[14–17]

The fore and hindwings of butterflies are serially homologous structures as are the serially homologous eyespots that can decorate each of these wings. Eyespots have been shown to have an adaptive deflective or intimidating function in interactions with predators[18–20] as well as a function in mate choice.[21] In some species, eyespots change markedly in size and coloration across different seasonal forms and these changes also appear to be adaptive to the different environments.[22] Eyespots also vary in number and morphology across species as well as across the fore and hindwings and dorsal and ventral surfaces of the same individual. Thus, in eyespots, we have a trait that is not only serially homologous but has known functional significance. Here I will focus on the evolutionary and developmental basis for eyespot number variation independently of the selective pressures that have led to such variation.

In butterflies, the maximum number of particular wing pattern elements is usually fixed within a family and does not exceed the total number of wing cells, i.e., the wing compartments bordered by wing veins. Among nymphalid butterflies, for example, the venation pattern is quite conserved and the total number of eyespots that can be present on the wings of these insects appears to be 8 (per wing surface) $\times$ 8 (total number of wing surfaces) $=$ 64. It is still unclear, however, how the eyespot developmental program became serially repeated in each of these wing compartments and how subsets of eyespots are able to display different morphologies.

Eyespots make up one of the symmetry systems of the Nymphalid Ground Plan (NGP) (Fig. 1A). This plan is a system of homologies that was put forth independently by Schwanwitsch and Süffert in the 1920s, and further elaborated by Nijhout.[23] It organizes most of the butterfly wing pattern diversity across the nymphalids and other butterfly lineages into the basal, central and border symmetry systems. Early experimental work (reviewed in Ref. 23) suggested that these systems appear to develop independently of each other on the wing. Eyespots are part of the border symmetry system, also called the border ocelli system.

Although the NGP was meant to represent the maximal number of repeated wing pattern elements that can develop on butterfly wings, it has also been suggested to represent an ancestral wing pattern. For instance, Nijhout[13] proposed that the row of border eyespots arose by compartmentalization by veins of a preexisting symmetry system composed of bands

Department Ecology and Evolutionary Biology, Yale University, 165 Prospect St, New Haven, Connecticut 06511.
E-mail: antonia.monteiro@yale.edu
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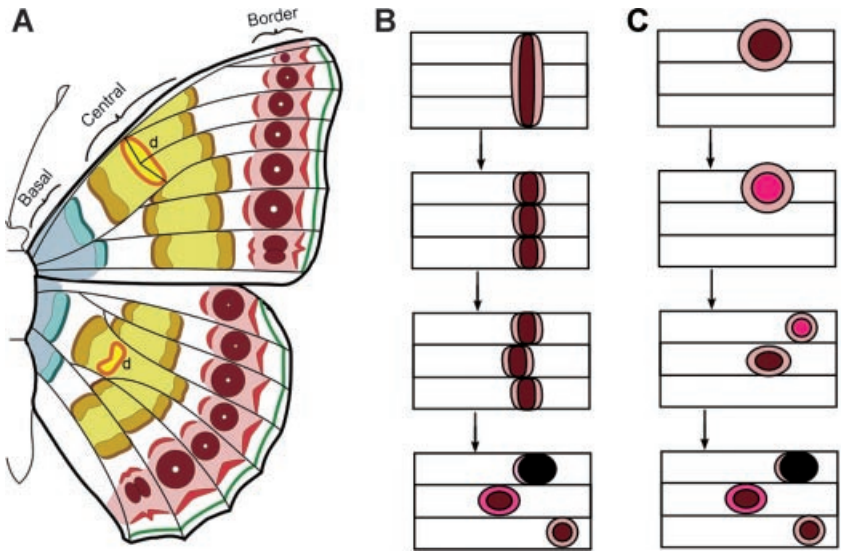

Figure 1. The Nymphalid Ground Plan (NGP) and two models for the evolution of eyespot number within the border symmetry system of nymphalid wings. **A:** The three main symmetry systems of the NGP (basal, central and border). The small d in the center of the wing marks the position of the discal-cell eyespot. **B:** Model 1—eyespots gradually evolve from a band of coloration that gets compartmentalized and individuated in each wing compartment. **C:** Model 2—eyespots appear sequentially on the wing. Panels A and B are modified from Ref. 13.

running along the entire anterior–posterior axis of the wing. This model (Fig. 1B), thus, clearly implies that the ancestral pattern for that row of serial homologues is that of eyespots present in each wing cell, that only later acquire their individuality or disappear from particular wing compartments. Below I will gradually introduce evidence that may support an alternative model (Fig. 1C). This model proposes that eyespots appeared originally as single units on the wing, and that the eyespot network was gradually co-opted into novel wing compartments until a row of eyespots was formed.

What is an eyespot?

In order to look at questions of eyespot evolution, it is perhaps best to start by describing what these structures are and what we know about their development. When Süffert and Schwanwitsch were trying to organize Lepidopteran wing pattern diversity, they initially identified eyespots as elements of the border ocelli system (see Ref. 23 and references therein). Several differently looking pattern elements, however, by virtue of their location and position relative to elements of the remaining symmetry systems, were considered members of the border ocelli system. Color patterns ranging from a simple dot to a splindle-shaped pattern with no concentric rings, to "V" or "M"-shaped pattern elements were considered homologous to "eyespots" (e.g. Fig. 2.19 in Ref. 23). The term "eyespot", however, appears to be most commonly associated with the type of border ocelli found in the largest Nymphalid subfamily, the Satyrinae. In the Satyrinae, "eyespots" usually consist of circular or quasi-circular structures, many containing a central white pupil and multiple concentric rings of colored scales.

For the purpose of investigating questions of eyespot evolution at the developmental level, it is perhaps most useful to use only a purely morphological description of these structures. This will allow us to investigate whether the final phenotype, which is the structure under selection, has a different developmental basis in different lineages, and whether, for instance, eyespots with multiple color rings evolve from simpler "spots". So, for now, I will call an eyespot a roughly circular pattern on the wing, with at least two concentric rings or with a single color disc and a central pupil.

Previous research on the genetic and developmental basis of eyespots in butterfly wings has mostly focused on experiments around two main nymphalid model species, *Junonia* (*Precis*) *coenia* (Nymphalidae: Nymphalinae) and *Bicyclus anynana* (Nymphalidae: Satyrinae). Both of these species have eyespots that consist of more than one concentric ring of color. In both species, transplantation during early pupal development of the group of cells at the center of the prospective eyespot pattern, the focus, results in the differentiation of a complete eyespot in the host epidermal tissue.[24,25]

Despite limited knowledge about the mechanisms that differentiate these focal cells from the surrounding tissue, we know that several genes are expressed in and around the focal cells during the late larval wing stage. In particular, the transcription factors Distal-less (Dll), Engrailed (En), Spalt

(Sal), Cubitus interruptus (Ci) and the receptors Notch (N) are expressed in both *Junonia* and *Bicyclus* foci, whereas the receptor Patched (Ptc), also expressed in foci, and its ligand Hedgehog (Hh), expressed in a region flanking the foci, were additionally visualized in *Junonia* wings.[26–30]

Later in development, during the early pupal stage, a known signaling ligand, Wingless (Wg) and an activated signal transducer (phosporylated pSmad) from the Transforming Growth Factor-β (TGF-β) signaling pathway, are expressed in the eyespot foci of *B. anynana*.[29] Subsequently, several transcription factors become expressed in concentric rings around the foci suggesting that they may be responding directly to one or both candidate morphogens (Wg and Tgf-β proteins) and defining the cellular domains that will later produce different color pigments[27,31,32] (Fig. 2). These data indicate that eyespots with concentric rings of coloration originate from signals produced in the differentiated group of cells at the center, the focus.

Despite similarities in the early development of *Junonia* and *Bicyclus* eyespots, there are some relevant morphological differences between the adult patterns. *Junonia* hindwing eyespots, for instance, don't display a clear pupil at their center (Fig. 3A, compare with Fig. 2E) despite displaying the focal marker genes during larval wing development. These morphological differences in the adult wing pattern indicate that we should not rely on the presence of a pupil in order to score a pattern with concentric circles as being produced by signaling from a group of central cells.

In many nymphalid species, moreover, patches of a single color appear along the margin of the wings and display neither pupils nor concentric rings of coloration (e.g. in *Idea lynceus*, a basal nymphalid; Fig. 3B). One possibility is that these patches are simplified eyespots, either displaying an enlarged focus or a single ring of color, produced in response to signals from a smaller central focus. There is, however, an alternative to these two possibilities, discussed in the next section.

In summary, *Junonia* and *Bicyclus* eyespots differentiate in response to signals produced at their centre whereas species from more basal Nymphalid lineages display spots, rather than eyespots, that may or may not be produced via a similar developmental process, and may or may not represent early morphological stages of eyespot evolution.

Basal butterfly families to the nymphalids (Pieridae and Papilionidae) appear to produce spots and eyespots in different ways

Recent work on a butterfly basal to the nymphalids, *Pieris rapae* (Lepidoptera: Pieridae), with two patches of black coloration on its wings (Fig. 3C), revealed that these patches did not express any of the genes associated with focal cells in the larval wing developmental stage, or signaling from the center during the pupal stage.[29] An alternative model for patch production was instead proposed for this species. Here, signaling from the wing margin, followed by interpretation of the signal along an anterior–posterior band of cells perceiving threshold concentrations of this signal would produce a continuous stripe of gene expression some distance from the margin. This stripe-gene would then be repressed or activated in specific wings compartments by other transcription factors, resulting in the appearance of "spots".[29] Ongoing work on eyespot-like patterns on another basal butterfly, from the Papilionidae (Fig. 3E), suggests differences in process there as well (P.M. Brakefield, personal communication). Basal lineages to the nymphalids appear, thus, to have independently invented other mechanisms to produce spots of color on their wings, whereas eyespots, produced with the previously described genes, appear to have evolved either with the Nymphalidae or in the lineage ancestral to both the Nymphalidae and its two sister lineages, the Riodinidae and Lycaenidae (Fig. 3E).

Eyespot evolution, evidence outside the nymphalids

Eyespots with concentric rings of coloration appear also in other Lepidopteran lineages, basal to the butterfly superfamily.[33] In these moth lineages, eyespots appear as single units on the wing in a more central position and are called discal-cell eyespots (see Figs. 1A and 3D). Because of their more central location on the wing, it can be argued that these eyespots are not homologous to any of the border eyespots

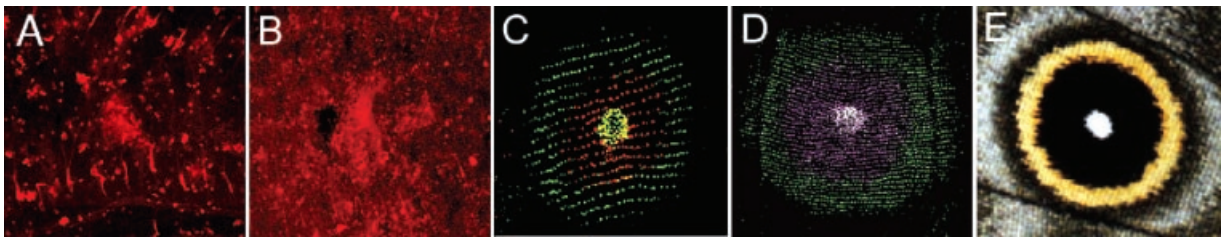

Figure 2. Genes expressed in the eyespot field during the pupal stage in *Bicyclus anynana*. **A:** pSmad (from the TGF-β signaling pathway), **B:** Wingless, **C:** Distal-less (red) and Engrailed (green), and **D:** Spalt (purple) and Engrailed. **E:** The corresponding eyespot.

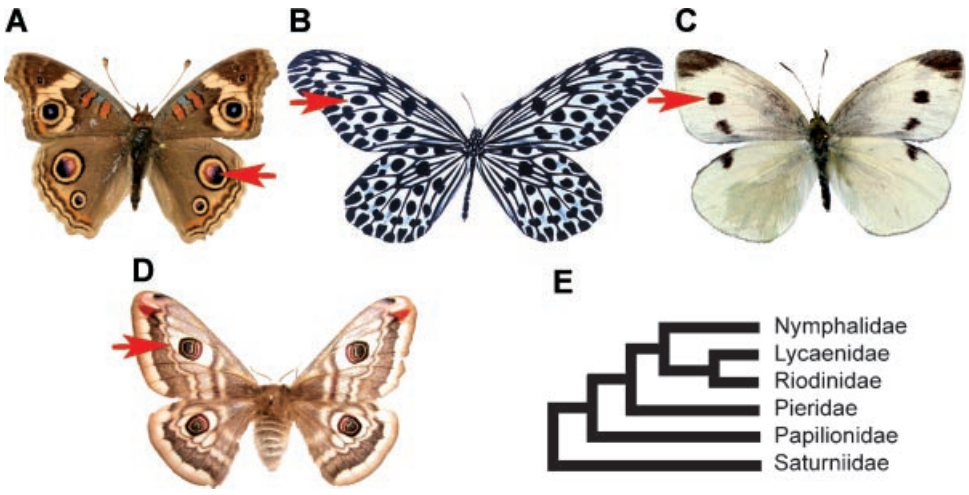

Figure 3. Eyespots and patches of color in different families of butterflies and moths. **A,B:** *Precis coenia* and *Idea lynceus* are both Nymphalidae. **C:** *Pieris rapae* (Pieridae). **D:** *Saturnia pavonia* (Saturniidae). Arrows indicate structures mentioned in the text. **E:** Relationship of butterfly lineages to saturniid moths.[33]

present in nymphalid wings and have originated via independent mechanisms. Investigations into the development of discal-cell eyespots in saturniid moths (Fig. 3D), however, revealed that these patterns with concentric rings of coloration share at least two of the focal cell marker proteins, *Distal-less* and *engrailed*, present in *Junonia* and *Bicyclus*,[29] suggesting that these eyespots may be process homologous[34] to nymphalid eyespots with concentric rings. The presence of eyespots in lineages outside the butterflies can signify two things: (1) that the developmental circuitry involved in building eyespots predates the origin of the Nymphalidae, first appearing deployed in a single central position on the wing, and later repeating and duplicating by multiple co-option events within the nymphalid lineage of butterflies, or (2) that the eyespot developmental program within the nymphalids was build de-novo, by reusing at least two of the same genes as those used by saturniid moths. Additional comparative work using a larger set of genes known to be expressed in nymphalid eyespot focus differentiation as well as in later stages of eyespot development, may start favoring one of these alternative hypotheses over the other. In addition it will be important to sample a broader sample of saturniid and other basal moth lineages with single eyespots, e.g., Hepialoidea, etc,[33] to verify whether those eyespots also share a common developmental basis.

Eyespot number variation in extant nymphalids—mutants

Spontaneous[35,36] and X-ray-induced mutations[12] can alter the number of eyespots that appear on the wing surfaces, producing substantial departures from the wild-type pattern (Fig. 4). For instance, the spontaneous mutation *Missing* represses the differentiation of two eyespot foci on both the fore and the hindwing in a dose-dependent fashion.[37] The spontaneous mutation *Spotty*, on the other hand, promotes the differentiation of these same two signaling centers but only on the forewing.[37,38] These two mutations, and the many others obtained through X-ray mutagenesis (Fig. 4) highlight the ability of genomic regions in current day nymphalid species to shut down or turn on the eyespot developmental network in only certain regions of the wing, with no effect on the development of the flanking eyespots.

These data support the idea that the eyespot developmental program consists of a modular network of interacting genes that can be induced by switching on a small set of genes at the top of the network, just as in the eyeless gene network [39]. Mutations such as *Missing* and *Spotty*, acting as molecular ''switches'', may represent modular cis-regulatory elements of genes at the top of the eyespot developmental module (as discussed below), responsible for activating the network in specific regions of the wing. It is also possible that these ''switches'' represent mutations in structural genes. For instance, selector genes expressed in only certain sections of the wing. The selectors would interact with genes from the eyespot network but would affect only the eyespots developing in those wing sectors.

Independently of what genomic regions explain eyespot number variation, what remains especially unclear is when these regions originated. Is the ancestral condition for the eyespot serial homologues one where a single or a few eyespots appear on the wing due to the appearance of mutations such as *Spotty*, and where the complete row of eyespots is put together in steps by the appearance of additional similar mutations in the genome? Or, did eyespots

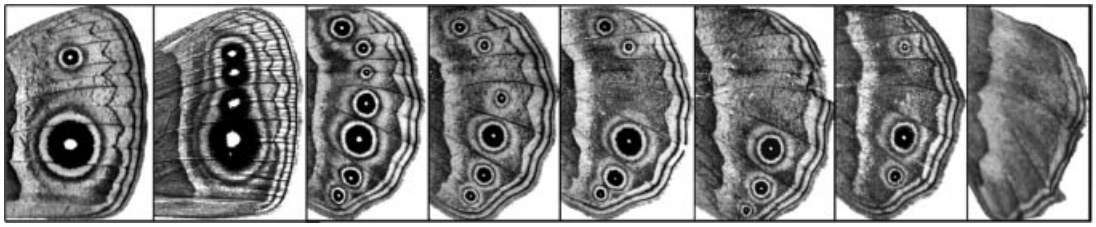

Figure 4. Ventral wing pattern of wild-type and eyespot-number mutants in *B. anynana*. From left to right: wild-type forewing, *Spotty*, wild-type hindwing, eyespots 3 and 4 reduced, *Missing*, eyespots 1, 2, 3 and 4 absent, eyespots 1, 3, 4, and 7 absent, and all eyespots absent.

appear as a cohesive unit of similarly looking structures, one per wing cell, resembling the proposed Nymphalid Ground Plan, that gradually gained separate regulatory control? In the latter option, did the separate regulatory control originate through the appearance of genomic regions such as *Missing* and *Spotty* that shut down and activate, respectively, the eyespot program in only certain regions of the wing? I suspect that we won't know the answers to these questions until we use phylogenetic comparative methods across a wide range of species and determine the most-likely wing patterns for ancestral and derived clades of species.

The evolution of serial homology: examples from other systems

There are a few examples from other systems that support each one of the two hypotheses of eyespot evolution. The evolution of arthropod limbs support the model where serial homologues appear simultaneously as multiple undifferentiated units repeated throughout the body that are later removed or modified in certain segments. Examples from the fossil record[40] and from experimental comparative studies across arthropods,[41,44] revealed that limbs first appeared as identical repeated structures that later acquired their unique morphologies along the anterior–posterior axis of the body (e.g. walking and feeding appendages) and whose development was also repressed in certain segments (e.g. in insect abdomens),[41–44] or de-repressed in certain lineages of Lepidoptera that develop abdominal appendages during the larval stage.[45] This modulation of the limb developmental program appears to be controlled largely by the hox selector genes, whose expression domains differ in the different sectors of the anterior–posterior body axis.[46]

The pair-rule gene *fushi-tarazu* (*ftz*), and its involvement in insect segmentation, supports the model where serial homology appears in a sequential fashion by the co-option of genes into novel domains. *ftz* is required for proper segmentation in *Drosophila*, and is expressed in a repeated pattern of seven stripes, marking every second segment.[47] In a more-basal grasshopper lineage, however, *ftz* has a single broad stripe of expression and is suggested to act as a homeotic gene, rather than as a segmentation gene.[47,48] ftz may have lost its proposed homeotic function and gained a pair-rule function by evolution of its protein sequence, but novel cis-regulatory domains also had to evolve in order for *ftz* to be expressed in a novel pattern of seven stripes. So, in this case, and assuming that the grasshopper expression pattern represents a basal insect *ftz* expression pattern, evolution of a repeated pattern of gene expression was acquired secondarily in insect evolution.

The evolution of modular gene regulation

even-skipped (*eve*) is another pair-rule gene that is expressed in a serially repeated pattern of seven stripes, similarly to *ftz*, but with a more-complex regulation. Much work on *cis*-regulatory control and modular mechanisms of gene regulation has focused on *eve*.[49,50] Despite the gene's regular expression in seven stripes, single stripes or small sets of stripes of *eve* expression are regulated independently by separate *cis*-regulatory elements (CREs). Although we now have a better mechanistic understanding of the CREs responsible for positioning each stripe during *Drosophila* embryogenesis, fundamental questions regarding the evolution of *eve*'s modular regulation remain unresolved. For instance, it is still unclear what the ancestral expression pattern for this gene was, both in basal insect and basal arthropod lineages. *eve* appears to have a rapidly evolving pattern of gene expression ranging from gap-like, pair-rule-like or segment-polarity-like.[51] In addition, little is known about *eve*'s CREs in lineages other than *Drosophila*. This makes it difficult to estimate the early evolutionary steps of *eve* regulation that lead to the modular regulation observed in *D. melanogaster*. Did a single CRE position this gene in a single stripe, followed by the appearance of novel CREs that led to the novel expression of eve in additional stripes? Or was it the case that *eve* appeared originally in a pattern of seven stripes, all controlled by a single CRE, that later duplicated and diverged, allowing each stripe to acquire independent regulation? One of the difficulties of addressing these questions using the comparative method involves having to deal with lineages with different number of segments and with different

modes of accruing segments,[52] e.g. while in some insect lineages the embryo divides into the final number of segments by the simultaneous expression of repeated stripes of the segmentation genes (e.g. *Drosophila*), in most other lineages, segments are added sequentially.[53]

With nymphalid butterfly eyespots, we have a system where the study of evolution of serial homology is greatly simplified due to the conserved venation system and homology of compartment areas where eyespots appear, disappear and are modified on the wing. Additionally, one of the proposed models for the modular control of butterfly eyespots[12] is largely based on the work developed around *eve* and explains not only mutations with large effects that control the appearance and disappearance of subsets of eyespots (Fig. 4) but also gradual and independent modifications to the eyespot module in different parts of the wing shown to occur via artificial selection experiments.[54]

This model proposes that eyespot network genes that operate early in the eyespot developmental cascade may have a modular cis-regulatory control region so that if mutations disrupt one of these modules only a single eyespot or a subset of eyespots are truncated in their development. This can happen if the transcription factors that bind to and activate that particular cis-regulatory module in a particular area of the wing are different from the transcription factors that activate the other modules in other regions of the wing. The uneven distribution of transcription factors on the wing blade is likely to happen as a result of vein patterning mechanisms, as observed during *Drosophila* wing development.[55] It is very likely, thus, that the butterfly wing is subdivided in several smaller and perhaps overlapping compartments defined by the expression of particular selector genes, allowing each wing compartment, or groups of adjacent wing compartments to have a separate ''genetic'' identity or genetic combinatorial code. These selector genes can then also be used to modulate the eyespot developmental network in the different sectors of the wing, as long as there are functional binding sites for these selectors in regulatory sequences of genes belonging to the eyespot network. Polymorphisms for these binding sites, for instance, could have provided the standing genetic variation that was used to gradually change eyespot size in one of the dorsal wing eyespots in an independent direction relative to the size of another eyespot on the same wing surface.[54] This model presents an alternative framework (as well as a molecular framework) for thinking about possible mechanisms of modular control of serially repeated eyespots, relative to that described in Fig. 1B but, just as in the *eve* work, does not address how this level of complexity would have evolved in butterflies.

Can eyespot individuation decrease as well as increase through time?

Many butterflies have eyespots that display different morphologies in different sectors of the wing, whereas other species have eyespots that all look fairly alike. Is eyespot divergence increasing or decreasing with time? It is possible to imagine two separate scenarios, dependent on how eyespot number evolution proceeded in the Lepidoptera, that support both an increase as well as a decrease in eyespot morphological individuation (see Fig. 5). If the row of eyespots originated simultaneously on the wing, by the compartmentalization of a continuous banding pattern, for instance, then these structures can only increase their average degree of individuation with evolutionary time (Fig. 5; H1). On the other hand, if eyespots get gradually co-opted into novel wing compartments on the wing (Fig. 5; H2), the eyespot program that may have evolved a particular morphology at the original wing location, under the control of a subset of selector genes, when co-opted into a novel location may revert to a more primitive

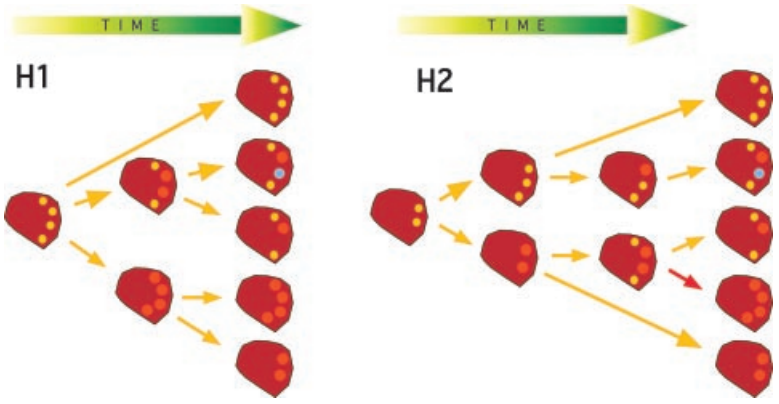

Figure 5. A graphical representation of the two hypotheses for eyespot evolution and individuality within Nymphalids. H1: average eyespot number decreases from the ancestral state (4 eyespots) to the current derived states (average 3.4 eyespots/species). H2: average eyespot number increases throughout evolution (from 2 eyespots to the same 3.4 eyespots/species). Eyespot individuality can increase or decrease (transition marked by red arrow) depending on different evolutionary scenarios.

(non-modified) morphology. In this case, when we currently see identical looking eyespots on a wing, this could represent "cryptic" instances of convergent evolution (see instance marked by a red arrow in Fig. 5).

Irrespective of how eyespot number evolved in the Lepidoptera, it is likely that the majority of the eyespot network genes still function today in a similar fashion across the different sectors of the wing. This inevitably leads to positive phenotypic and genetic correlations among eyespots that are commonly found in population genetic studies[56,57] or through the course of artificial selection experiments, when selection on a single eyespot trait leads to correlated changes across all eyespots.[58–60]

Conclusions

The repetition and modification of modules is a common feature in the design of complex organism. Exploring the evolution of serial homology of eyespots in nymphalid butterflies is likely to contribute to our understanding of the evolution of repeated structures and their individuation in organisms. In order to accomplish this task, however, it is important to progress on several fronts. Reconstructing the ancestral wing pattern of nymphalid butterflies and of more basal lineages is an important first step to understand when the serially repeated eyespots appeared. Then it will be interesting to ask what selection pressures and ecological circumstances have led to the current day variation in eyespot number or to an overall increase in eyespot number, if H2 is shown to be correct. Recent work on lepidopteran paper models with a variable number of eyespot patterns[62] has shown than predators are deterred by designs carrying more eyespots. Additionally, in order to understand "how" eyespots are able to appear and disappear from the wing and acquire their separate identities, it is important to pursue more mechanistic and molecular studies. For instance, it is critical to continue dissecting the eyespot network by performing both gene expression studies in a broad range of species, as well as detailed functional studies in a smaller subset of species.

Gene expression studies are extremely valuable because they will highlight which components of the eyespot network are present across all eyespot-bearing Lepidoptera. If these components vary from species to species, even within nymphalids, then a closer phylogenetic examination of the differences may indicate important steps in network evolution. Currently, four signaling pathways and several transcription factors have been implicated in eyespot development in nymphalids. It is possible, although unlikely, that the earliest eyespot precursor network had this level of complexity. Components of the network were probably added sequentially.

Figure 6. Specimens from different insect orders containing eyespots. Clockwise from top left corner: *Chrysochroa ocellata* (Coleoptera, Buprestidae), *Anatis borealis* (Coleoptera, Coccinellidae), *Fulgora lanternaria* (Hemiptera, Fulgoridae), *Papilio xuthus* larva (Lepidoptera, Papilionidae), *Alaus oculatus* (Coleoptera, Elateridae), *Pseudocreabatra ocellata* (Mantodea, Mantidae). According to the Tree of Life web project, Mantodea represent the most basal lineage, sister to the other lineages represented here. Hemiptera is then sister to the lineage composed of Coleoptera and Lepidoptera. *Papilio xuthus* larval picture courtesy of Ryo Futahashi.

For instance eyespots may have started as simple spots that later acquired multiple rings. Comparative studies are needed across both butterfly and moth lineages to determine whether the same network is being used in these taxa. Detailed functional studies, with putative network genes, are also important because a simple description of the gene expression patterns will not be enough to infer connectivity among the elements of the network, nor how these connections evolve though time. Understanding which genes are upstream in the network, for instance, will pinpoint likely candidates that may harbor the ''switch'' genomic regions, able to activate or shut down the complete eyespot developmental program.

On another front, work should be directed towards trying to identify the genes or genomic regions of mutants such as *Missing* and *Spotty* that control the appearance and disappearance of single eyespots or groups of eyespots on the wing. These mutations may represent modular regulatory or protein-coding sequences, flanking or embedded in the cDNA of important genes, that have a clear impact as modular regulators of the eyespot developmental program. These on–off ''switch'' genomic regions, once identified, will enable comparative work on the evolution of the serial homologues to be performed at the molecular level. We will finally be able to ask whether these modular control regions were gradually acquired during evolution, or whether they evolved from non-modular regions that initially positioned all of the eyespots simultaneously on the wing, and that later got ''fragmented'' (or duplicated) in order to start controlling specific eyespots or subsets of eyespots on the wing.

The great variability present in the border symmetry system of butterflies, the clear homologous venation system across nymphalid wings and the large number of extant species where comparative work can be performed, are all factors that make the serial eyespots an untapped resource to address the evolution of serial homology in organisms.

Finally, it is interesting to note that eyespots appear to have evolved as single units on the wings of other insect lineages such as in fulgorid hemipterans, praying mantids and some beetles, as well as on the larval cuticle of some lepidopteran larvae (Fig. 6). It should also be very interesting to study the developmental basis of those patterns to determine whether they are sharing the same developmental network as that of nymphalid and/or saturniid eyespots. Whatever the result, the answer is likely to provide some fascinating insights into the evolution of complex and novel structures. We may find, for instance, that there is a single ancient ''eyespot'' network, that functions both as a developmental and as an evolutionary module, i.e. a network of tightly interconnected genes that operates in a context independent fashion and that can be co-opted as a unit to novel locations on the wing many times independently.[61] Alternatively, we may learn that building a complex structure, such as an eyespot with multiple rings of color, by putting together a network with novel (or similar) gene combinations from scratch is not difficult to do. Whatever way eyespots are built in these insect lineages, the inescapable conclusion is that strong natural and/or sexual selection has been acting on the final bull's eye structure throughout evolution presumably because of its adaptive value, be it in butterflies, hemipterans or mantid's wings.

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