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Molecular Evolution: Breakthroughs and Mysteries in Batesian Mimicry

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Recent studies appear to overthrow the hypothesis that, in butterfly species exhibiting Batesian mimicry, a multi-gene complex or 'supergene' controls the multiple differences between mimetic and non-mimetic individuals, suggesting instead that near-perfect mimicry can be produced by a set of changes within a single locus, together with changes in the genetic background.

Mimicry has attracted the curiosity of biologists because it involves wonderful resemblances between unrelated species. Batesian mimics are palatable, undefended species that avoid predation by having evolved resemblances to unpalatable or defended 'model' species [1]. In several butterflies with Batesian mimicry, only some individuals are mimetic, and this polymorphism has allowed the genetic control of mimicry to be studied. The genetic control is interesting because mimicry involves multiple changes, including both wing patterns and wing and body colours, and even the presence or absence of hindwing tails, which seem unlikely to be controlled by a single gene. Mimicry is thus a complex adaptation. Surprisingly, genetic studies in several butterfly species have indicated that a single locus controls these complex traits [1]. To explain this, it was proposed that adaptive differences between mimetic and non-mimetic butterflies evolved in genes that control the different traits, and that these genes are in a closely linked genome region, allowing establishment in such species of a polymorphic multi-gene complex or 'supergene' [1]. New results [2,3] now suggest a modified 'multi-site' mimicry supergene in a butterfly, involving mutations in a just single large gene.

Whether the mimicry 'locus' is a single gene or a supergene including several different genes can be tested using genome sequence data, as follows. Both the multi-gene and the multi-site versions of the supergene hypothesis predict that the mimetic and non-mimetic alleles at the mimicry locus will be differentiated in sequence, as a result of evolution of suppressed recombination across the genome region in which the causal variants are located. In either case, a rough mimetic resemblance probably first arose by a single mutation. Such mutations can increase in frequency, but will often not spread throughout the population, because the model species are usually more conspicuous than non-mimics, increasing their rate of predation. If another mutation arises in the region, improving the mimicry, selection for reduced recombination may occur, because the combinations of both mimicry mutations, or both non-mimetic alleles, give high survival, whereas other combinations lead to imperfect mimics that are more conspicuous than non-mimics [4].

Suppressed recombination isolates the mimetic and non-mimetic alleles. Over time, the two types of alleles, mimetic and non-mimetic, will acquire new mutations that remain associated with the allele in which they arose, so that the two types become genetically differentiated, like geographically separate populations, or like an X and a Y chromosome. Importantly, many of these variants will not affect the mimetic patterns — the associations with the mimetic alleles are due solely to their evolutionary isolation within a non-recombining genome region. If a multi-gene supergene has evolved as outlined here, intervening genes not involved in controlling mimicry will therefore also be differentiated.

Two recent studies [2,3] studied Batesian mimicry in the butterfly *Papilio polytes*, identifying the genome region that includes the mimicry locus, and providing sequence data that can test the supergene hypothesis. Both studies conclude that a single gene is responsible for genetic control of mimicry, and not a linked complex including multiple genes. *P. polytes* includes multiple, regional mimetic forms that presumably differ in relation to the distribution of model species, and there are also several geographic races. Using the *alphenor* race, the first study [2] genetically mapped the control of mimicry to a single

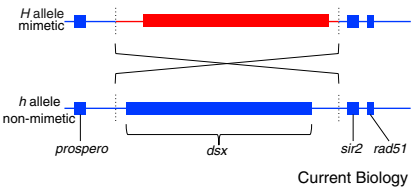

Figure 1. Structure of the mimetic (*H*) and non-mimetic (*h*) alleles of the butterfly *Papilio polytes*.
The diagram shows the chromosomal inversion distinguishing the *H* and *h* alleles (not to scale). Vertical lines indicate inversion breakpoints. Sequence divergence between *H* and *h* chromosomes is expected to be greatest within the bounds of the inversion, i.e. for *dsx* and the flanking intergenic DNA, while flanking regions and genes should be similar between the two types. Simplified from [2,3].

genome region including the developmentally important gene *doublesex* (*dsx*). The second study [3], using another race, *polytes,* again mapped mimicry to the *dsx* gene, also showing that multiple sequence variants in *dsx* differentiated wild-caught mimetic from non-mimetic individuals. Impressively, both studies provided evidence that the expression of *dsx* affects wing pattern formation (though the proposed mechanistic scenarios differ).

Both these studies [2,3] generated genome sequences of *P. polytes* individuals with mimetic (*H*) and non-mimetic (*h*) alleles, and assembled the roughly 100 kilobase *dsx* genome region. In both assemblies, the region surrounding *dsx* is structured into different haplotypes carrying either the *H* or *h* alleles, between which recombination appears to have been suppressed by an inversion spanning the *dsx* locus. The inversion does not extend far beyond *dsx*, suggesting that no extended supergene exists (Figure 1).

If the mimicry polymorphism has been maintained for a long time, divergence between *H* and *h* alleles could be high, relative to the rest of the genome. Indeed, comparing *P. polytes dsx* with other genes in the flanking recombining region, nucleotide diversity in *P. polytes dsx* is unexpectedly high, relative to divergence from another *Papilio* species, *P. canadensis* [2]. However, the individuals sequenced were not from natural *alphenor* race populations, so this test is not yet completely definitive; it should be applied to the *polytes* race data now available [3], and a closer relative than *P. canadensis* would also be preferable. Nevertheless, there is no sign of sequence divergence extending across flanking genes, so the results disprove the multi-gene supergene hypothesis. Instead, both new sets of sequence results suggest a multi-site supergene, with several mutations in a single large non-recombining gene, *dsx.* Strikingly, results from another mimetic butterfly, *P. dardanus*, also genetically mapped the mimicry locus to a region including no more than a few genes [5].

Looking more closely at the differences between the *P. polytes H* and *h* alleles reveals some intriguing patterns in their molecular evolution, illustrating the importance of extending the work to further investigate sequence differences. The *dsx* coding sequences from the two studies [2,3] can readily be aligned to provide reliable analyses of sequence divergence. A first surprise is that inter-race differences are large. Synonymous site divergence (K_S) between the *H* alleles (Figure 2) is 16%. This is comparable with the 14% divergence between butterfly species *Heliconius melpomene* and *H. erato*, which probably corresponds to about six million years of separation [6]. The mimicry alleles from the two races apparently share the same inversion, and their inter-race divergence should reflect the same separation time. However, K_S between the two races is only 8% for the *h* alleles (Figure 2).

Divergence between the *H* and *h* alleles is considerably higher — 26% for race *alphenor* and 29% in the *polytes* race — suggesting very old-established recombination suppression. As previously found [2], divergence between

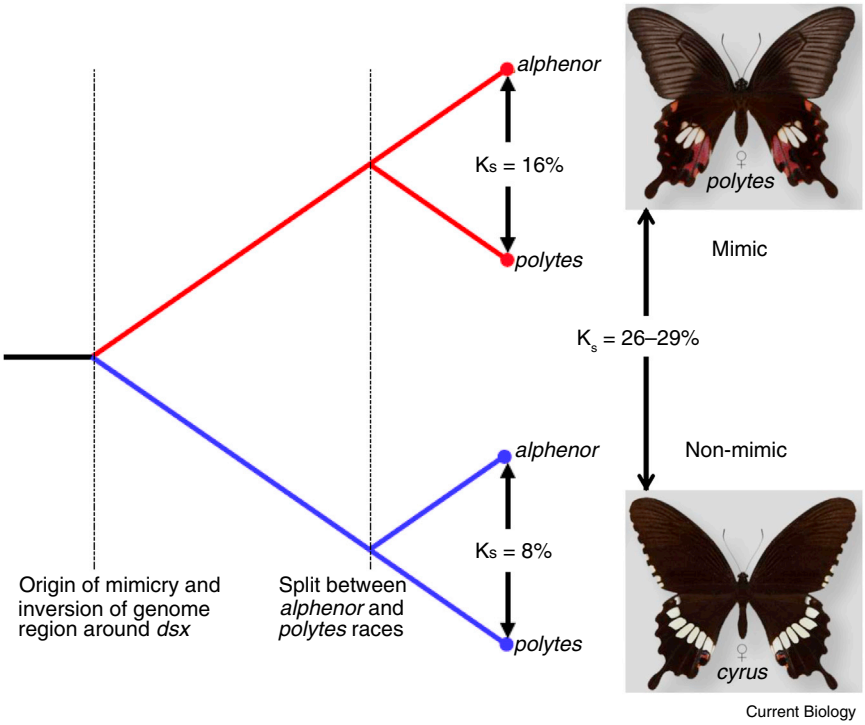

Figure 2. Schematic genealogy of the *dsx* gene controlling Batesian mimicry in the butterfly *P. polytes*.
The diagram is based on an initial split between non-mimetic and mimetic haplotypes that occurred at about the same time as the inversion spanning *dsx*, which suppressed recombination. A subsequent split into the two *P. polytes* geographic races *polytes* and *alphenor* then occurred, allowing inter-race differentiation of the mimetic and non-mimetic alleles. The actual estimates of synonymous site divergence (Ks values) between the two populations and haplotypes, calculated using the *dsx* coding sequence, are shown. Alignment gaps were excluded (but few such indels were found), but sites that were heterozygous in the individuals sequenced were; correction for such variants within the races would reduce the estimated extent of fixed differences between the two types of alleles, but at most by only a few percent [7]. We thank Krushnamegh Kunte (National Center for Biological Sciences, Bangalore, India) for the images of *P. p. alphenor*.

H and *h* alleles is greatest in exon 1 of *dsx*, consistent with the suggestion that mutations controlling mimicry may be located within it [2]. It is surprising, however, that a signal of the initial mutations is detected, as the great age of the isolation between the two allele types, allowing many other mutations to occur, should have over-written it.

It is a triumph to have identified the mimicry gene, and to have shown that it is a single locus, overthrowing the long-established multi-gene version of the supergene hypothesis. These new studies [2,3] illustrate how molecular evolutionary approaches now allow long-standing interesting biological questions, which have been inaccessible to study, to be revisited. As with all genome sequencing of non-model organisms, however, assembly is very challenging, particularly in polymorphic non-recombining genome regions. In this case, the puzzling results concerning the divergence of the different alleles suggest that the assemblies need very careful validation before important biological conclusions, such as a great age of the mimicry polymorphism, can be accepted. Validated assemblies and natural population samples should soon allow population genetic analyses to test for long-term balancing selection maintaining different alleles polymorphic at *dsx*.

The control of mimicry in *P. polytes* by *dsx*, perhaps including its immediately flanking region [3], and the similar findings in *P. dardanus* [5], raise very interesting questions about the evolution of the complex adaptation involved in mimicry. How can a single *P. polytes* gene control such developmentally different characters as colours and hindwing tails? The answer probably involves initial mutations producing rough mimetic resemblances to model species, and evolution later improving the mimicry through fixation of 'modifier' alleles (which could be alleles at unlinked loci). These modifiers must affect specific morphs (for instance, changing the colour of an initial rough mimic to make it more closely resemble its model species); if the non-mimetic form is also affected, the increased conspicuousness associated with the mimetic morphs would reduce the survival of the non-mimics, and the modifier allele would either be unable to spread in the population, or would drive the mimicry allele to fixation, abolishing the polymorphism [4]. It is even more mystifying to explain the evolution of the multiple different mimetic forms that are known within several butterfly species with Batesian mimicry, including both *P. polytes* and *P. dardanus*.

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Action: The Role of Motor Cortex Challenged

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The motor cortex is often considered the main controller for movement, but a new study shows that well-trained paw movements can be performed with equal precision after lesions of the entire motor cortex; the motor cortex is, however, required for learning a new task in naïve animals.

In the textbook version of motor control, the motor cortex holds a central position implemented via direct projections to the spinal cord. Is this view compatible with recent and older findings? A new study from the Ölveczky laboratory [1] challenges this view in very important aspects: it shows that, in a task requiring a rat to perform two sequential lever presses with a precise time interval, the rat performs the task in a stereotyped way with the same precision before and after a large lesion motor cortex and related areas of the frontal lobe. Clearly this means that the circuits producing the paw presses do not require the motor cortex and that they are not important for determining the precise time interval;

