

News & Views:

New butterfly genomes clarify mimicry evolution

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*For over 100 years it has been known that mimicry polymorphisms are often switched by simple Mendelian factors. Yet the physical nature of these loci has hitherto escaped characterization. The sequencing of two swallowtail butterfly (*Papilio*) genomes marks the latest episode of an extraordinary few years of discoveries in mimicry genetics.*

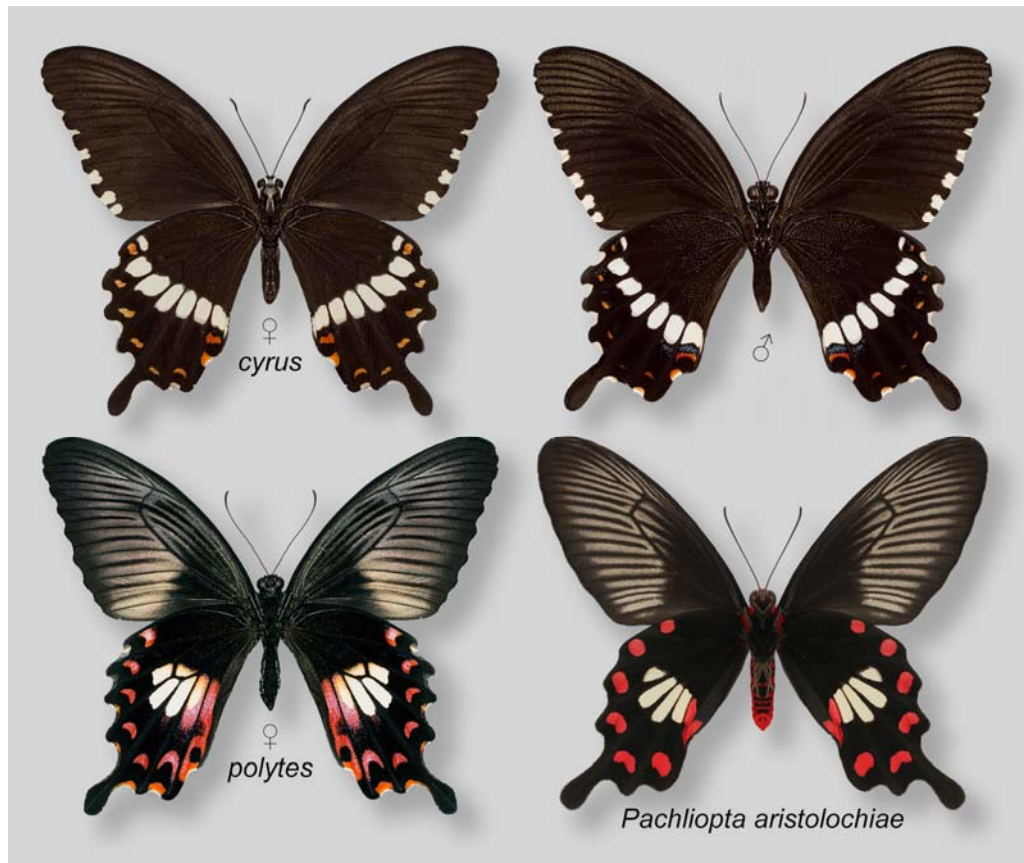


Figure 1. Mimetic and non-mimetic *Papilio polytes* and its noxious model

Top row: non-mimetic form and male; bottom row: mimetic female and the noxious model species, *Pachliopta aristolochiae*. (Krushnamegh Kunte © 2015).

Non-poisonous butterflies that mimic noxious species are "cheats:" their colour patterns lie about their toxicity. One example is the swallowtail butterfly *Papilio polytes*. Females occur either as a male-like, black-and white form which is non-mimetic, or as a red-spotted form that mimics a poisonous

species, *Pachliopta aristolochiae* (Fig. 1). On p. 405 of this issue, Haruhiko Fujiwara and colleagues report the genome sequences of *Papilio polytes* and a close relative, *P. xuthus*. They convincingly show that mimicry in *P. polytes* is effected by a single gene, *doublesex* (*dsx*)¹.

This work joins a flurry of recent genomic work on mimicry in *P. polytes* and other butterflies²⁻⁶, which together bring us much closer to understanding how mimicry works.

Characterization of a mimicry supergene

Decades ago, it was hypothesized that mimicry switch loci might consist of multiple, tightly linked genes, collectively acting as a "supergene"⁷. In 2014 the so-called *H* locus (the mimicry switch gene) of a Philippines population of *P. polytes* was shown to consist of an allele of *dsx* within a small ~130 kb inversion⁵. This finding was too bizarre to have been predicted: *dsx* is a highly conserved transcription factor involved in sex-determination of both protostomes and deuterostomes. How on earth could such an important gene be co-opted to twiddle with colour patterns late in the development of the wing of an adult butterfly? The finding was surprising in another way. Rather than a collection of genes being contained in the mimicry "supergene", it appeared that coding changes in and perhaps non-coding regions near *dsx* itself were responsible.

Last year's paper was astounding enough, but it is now evident that Fujiwara's group was working independently on the same problem in a different population of *Papilio polytes*. Indeed, they had already hinted that they had tracked the *H* locus to a sex-determination gene⁸. The current paper¹ raises the bar for mimicry publications: the *dsx* locus is characterized in much greater detail to show that both inversion breakpoints are located within a few base pairs of each other. In addition, two new *Papilio* whole genome assemblies, as well as fosmid clone sequences covering the *dsx* region, are thrown in for good measure. For the first time, the function of *dsx_H* is proved in mimicry: small interfering RNAs (siRNA) designed to knock down *dsx_H* repressed mimetic phenotypes in the mimetic form; in contrast, *dsx_h* knockdown failed to suppress mimetic patterning. Fujiwara et al. infer that shows that the "default" male-like colour pattern is determined by genes elsewhere in the genome, with little involvement from *dsx*. The *dsx_H* allele acts by switching those genes to make a different female pattern.

The system's surprising flexibility may be due to modularity within *Dsx*; only some peptide domains are highly conserved and required in sex-switching and DNA binding. Another layer of modularity is due to alternative splicing of *dsx* transcripts: in *Papilio* there is a single male isoform, but three different

female isoforms, each of which can be expressed differentially, between body and wings, for example⁵. These findings give a glimmer of understanding as to how the central function of *dsx* in primary sex determination, such as ovary and testis development, may be detached from later activity in colour pattern development of the adult wing surface⁵.

The *dsx_H* allele is the most highly divergent 100 kb region of DNA (~ 25%) in the whole *P. polytes* genome. This nucleotide divergence is presumably maintained by balancing selection acting on multiple sites in *dsx*, while its private inversion inhibits recombination. There are around 20 or so coding differences between *dsx_h* and *dsx_H* in both India and Japan. However, only five of these differences are specific to *dsx_H* compared with other non-mimetic Lepidoptera¹. The other coding differences may represent real differences in patterning between The Philippines and Okinawa, or they may be hitch-hiking variants of little importance. The many non-coding differences likely include some that have non-coding RNA or cis-regulatory functions.

Controversies about mimicry

This year marks the centenary of a long-standing genetic controversy about mimicry. Not long after the rediscovery of Mendel, in 1915 Reginald Punnett⁹ demonstrated that mimicry in *P. polytes* and other *Papilio* was switched simply by a single dominant allele, *H*. In the first ever population genetic analysis of natural selection, Punnett showed that directional selection should quickly fix polymorphic variants. He concluded that long-lived polymorphisms, as in *P. polytes*, could not be under selection, and that mimicry arose as a neutral large-effect mutation of the *H* locus.

Mimicry became a *cause célèbre* of the Modern Synthesis of Darwinism and Mendelism in the 1930s-1970s. Ronald A. Fisher devoted a whole chapter of his ground-breaking book¹⁰ to mimicry, to promote Darwinian gradualism and rebut the earlier Mendelians' ideas. Punnett did not know about balancing selection, which Fisher discovered soon after. Fisher proposed that mimetic polymorphisms could be stable due to frequency-dependent selection: as "cheats" became common, their mimicry would be rumbled by predators and the advantage lost, leading to equilibrium. He argued that perfect mimicry necessarily involved gradual adjustment of many pattern elements, surely requiring multiple genetic loci. Polymorphism in *P. polytes* and other mimics was switched by a single locus, but the system had to depend on multiple interacting "modifiers" scattered throughout the rest of the genome to fine tune dominance and control by the switch locus itself, giving seamless transitions between adaptive forms. In *Papilio*, the *H* locus would thereby be able to transition between male-like and mimetic females without disadvantageous intermediates.

Later, crosses of *P. polytes* and other *Papilio* species by Cyril Clarke and Philip Sheppard largely supported Fisher's views on multifactorial inheritance, but added the new idea that some of the loci may be prevented from recombining via tight linkage in a "supergene." In *Papilio dardanus* and *P. polytes*, Cyril Clarke and Philip Sheppard showed that crosses between geographically distant populations, but not local populations, tended to produce intermediate and poorly adapted forms¹¹. They concluded that linked sites and unlinked modifiers elsewhere in the genome diverged in different localities, and were being perfected for interaction only with the local switch gene, as suggested by Fisher. While Clarke & Sheppard provided excellent evidence for involvement of multiple genetic changes in the mimicry switches, in those pre-molecular-marker days, the mode of action of mimicry "supergenes" was still murky.

In the last couple of years, we've learned a lot more. But we're still ignorant of which of the many differentiated sites in the *dsx_H* region are effectors of mimicry. Furthermore, there are intriguing expression differences for *dsx_h*, *dsx_H*, and their isoforms, and even for some genes just outside the *dsx* inversion^{1,5}. We are only beginning to understand how *dsx*-based sex determination in Lepidoptera differs from that in *Drosophila*¹², and we know none of the downstream targets of *dsx* in colour pattern pathways. The latest findings in *Papilio polytes*^{1,5} revolutionize our understanding of the genetics and evolution of mimicry, but we still have a way to go before we'll fully grasp this extraordinary adaptation.

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