New genomes clarify mimicry evolution

James Mallet

For over 100 years, it has been known that polymorphic mimicry is often switched by simple mendelian factors, yet the physical nature of these loci had escaped characterization. Now, the genome sequences of two swallowtail butterfly (*Papilio*) species have enabled the precise identification of a locus underlying mimicry, adding to unprecedented recent discoveries in mimicry genetics.

Non-poisonous butterflies that mimic noxious species use color patterns to deceive predators. One example is the swallowtail butterfly *Papilio polytes*. Females occur either as a male-like, black-and-white form that is non-mimetic or as a red-spotted form that mimics a poisonous species, *Pachliopta aristolochiae* (**Fig. 1**). On page 405 of this issue, Haruhiko Fujiwara and colleagues report the genome sequences of *P. polytes* from Okinawa and a close relative, *Papilio xuthus*. They show that mimicry in *P. polytes* is effected by a single gene, *doublesex* (*dsx*)[1]. This work joins other recent genomic studies of mimicry in *P. polytes* and other butterflies[2–6], 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' (refs. 7,8). In 2014, the *H* locus (the mimicry switch locus) of a Philippines population of *P. polytes* was shown to consist of an allele of *dsx* within a small, ~130-kb inversion[5]. This finding was unexpected, as *dsx* encodes a highly conserved transcription factor involved in the sex determination of both protostomes and deuterostomes. How could such an important gene be co-opted to alter color patterns late in the development of the wing of an adult butterfly? It was also surprising that a single gene, rather than a collection of genes contained in a supergene, appeared to be responsible.

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In 2013, Fujiwara and colleagues hinted that they had tracked the *H* locus in *P. polytes* to a sex-determination gene[9]. Now, Fujiwara and colleagues[1] have characterized the *dsx* locus in much greater detail to show where both inversion breakpoints are located. In the course of this work, they have reported two new *Papilio* whole-genome assemblies as well as fosmid clone sequences covering the *dsx* region. They have also carried out the first functional test of the mimetic allele of the *dsx* locus, *dsx*(*H*): small interfering RNAs (siRNAs) that knocked down *dsx*(*H*) repressed mimetic phenotypes; in contrast, knockdown of the non-mimetic *dsx*(*h*) allele had no effect. Nishikawa *et al.* infer that the 'default' male-like color pattern is determined by genes elsewhere in the genome, with little involvement from *dsx*. The *dsx*(*H*) allele is presumed to act by switching those other genes to make a different pattern in mimetic females.

The system's surprising flexibility may be due to modularity within the Dsx protein; only some peptide domains are highly

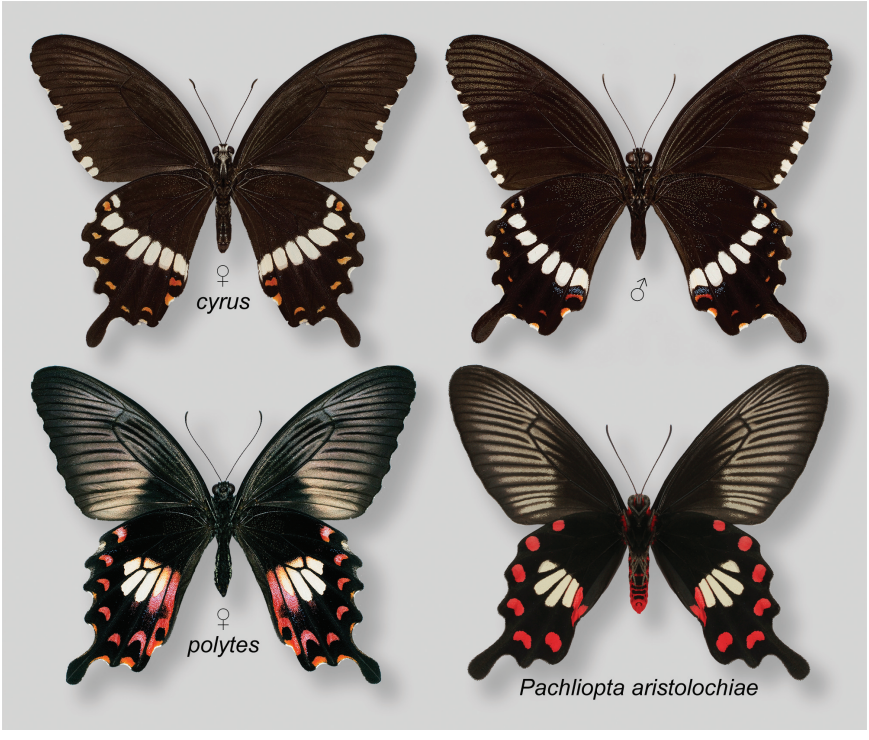

Figure 1 Mimetic and non-mimetic *P. polytes* and the noxious model for the mimetic form. Top row, the non-mimetic form and a male. Bottom row, a mimetic female and the noxious model species *P. aristolochiae*. (The image was provided courtesy of Krushnamegh Kunte.)

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 there are three different female isoforms, each of which can be differentially expressed, such as between body and wings[5]. These findings suggest how the central function of *dsx* in primary sex determination, such as ovary and testis development, can be detached from later activity in color pattern development of the adult wing surface[5].

The *dsx*(*H*) allele is the most highly divergent 100-kb region in the whole *P. polytes* genome. This divergence is presumably maintained by balancing selection acting on multiple sites in *dsx*, while the private inversion of the region inhibits recombination. There are around 20 or so coding differences between *dsx*(*h*) and *dsx*(*H*) in *P. polytes* populations in both the Philippines and Okinawa, but only 5 of the differences are specific to *dsx*(*H*) compared with *dsx* in other non-mimetic Lepidoptera[1]. Sequence differences between the Philippines and Okinawa may represent real differences in patterning between populations, or they may be hitchhiking variants of little importance. There are also many noncoding differences, some of which may have a regulatory function.

Controversies about mimicry

This year marks the hundredth anniversary of a long-standing genetic controversy about mimicry. In 1915, Reginald Punnett[10] demonstrated that mimicry in *P. polytes* and other *Papilio* species was switched simply by a single dominant allele, *H*. Punnett also 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 the result of a neutral, large-effect mutation.

Later, Ronald Fisher[11] proposed a selection-based alternative—that the mimetic polymorphism was under frequency-dependent selection: as mimics became common, the deceptive mimicry would be unmasked by predators and the advantage lost, leading to equilibrium. Although polymorphism in *P. polytes* was switched by a single locus, Fisher argued that the system must 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 supported Fisher's views[7,8]. Although Clarke and Sheppard provided experimental evidence for the involvement of multiple genetic changes in the mimicry switches, in those days before molecular markers, the mode of action of mimicry supergenes was still murky.

The recent reports on mimicry in *P. polytes* highlighted here[1,5] reveal much about the physical nature of the mimicry switch locus, but we still do not know 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, that may contribute to color pattern switching[1,5]. We are only just beginning to understand how *dsx*-based sex determination in Lepidoptera differs from that in *Drosophila melanogaster*[12], and we still do not know any of the downstream targets (the modifiers) of *dsx* in color pattern pathways. The latest findings in *P. polytes*[1,5] revolutionize our understanding of the genetics and evolution of mimicry, but we have a way to go before we fully grasp how this extraordinary adaptation works.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Nishikawa, H. *et al. Nat. Genet.* **47**, 405–409 (2015).
2. Reed, R.D. *et al. Science* **333**, 1137–1141 (2011).
3. Martin, A. *et al. Proc. Natl. Acad. Sci. USA* **109**, 12632–12637 (2012).
4. Gallant, J.R. *et al. Nat. Commun.* **5**, 4817 (2014).
5. Kunte, K. *et al. Nature* **507**, 229–232 (2014).
6. Timmermans, M.J.T.N. *et al. Proc. R. Soc. Lond. B* **281**, 20140465 (2014).
7. Clarke, C.A. & Sheppard, P.M. *Phil. Trans. R. Soc. Lond. B* **263**, 431–458 (1972).
8. Turner, J.R.G. in *Mimicry: The Palatability Spectrum and its Consequences* (eds. Vane-Wright, R.I. & Ackery, P.R.) 141–161 (Academic Press, 1984).
9. Nishikawa, H. *et al. Sci. Rep.* **3**, 3184 (2013).
10. Punnett, R.C. *Mimicry in Butterflies* (Cambridge University Press, 1915).
11. Fisher, R.A. *The Genetical Theory of Natural Selection* (Clarendon Press, 1930).
12. Nagaraju, J., Gopinath, G., Sharma, V. & Shukla, J.N. *Sex Dev.* **8**, 104–112 (2014).

Transcriptional mimicry by tumor-associated stroma

Hoon Kim & Roel G W Verhaak

Recent molecular classification of colorectal cancer (CRC) has identified a poor-prognosis transcriptional subtype associated with mesenchymal traits. New studies used CRC transcriptomic data to show that tumor-associated stroma mimics the gene signature of epithelial-to-mesenchymal transition (EMT) and found no evidence for EMT of colorectal tumor cells.

Molecular classification of cancer based on gene expression profiling provides an attractive alternative to traditional histopathology-based methods. Recent studies have sought to address the heterogeneity of CRC, leading to the development of three CRC classification systems based on gene expression[1–3]. Each of the classification systems included a transcriptional subtype associated with adverse patient outcomes and characterized by a gene signature that reflected a stem cell–like or mesenchymal cell–like nature, and this subtype was referred to as stem/serrated/mesenchymal (SSM). It was hypothesized that the SSM subtype reflects traits of EMT[4], whereby cancer cells of epithelial origin acquire mesenchymal features that are associated with a higher likelihood of invasion and metastasis. In this issue of *Nature Genetics*, independent studies by Claudio Isella and colleagues[5] and Eduard Batlle and colleagues[6] follow up on these recent findings to show that the transcriptional footprint of tumor-associated stroma weighs heavily on the SSM expression class, a finding that has profound implications for our understanding of the tumor-enhancing role of the microenvironment.

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Unlocking the tumor stromal transcriptome

Two orthogonal approaches were deployed to uncover the role of tumor stroma in the