CellPress

Current Biology
Dispatches

Ecological Genetics: A Key Gene for Mimicry and Melanism

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http://dx.doi.org/10.1016/j.cub.2016.07.031

Mimicry and melanism in Lepidoptera provided the first convincing examples of natural selection in action. Genetic analysis has now shown that, surprisingly, mimicry in *Heliconius* butterflies and melanism in peppered moths are switched at precisely the same gene: *cortex*.

The major revolution in Charles Darwin's '*On the Origin of Species*' was the proposal that evolutionary change took place by natural selection. The '*Origin*' was highly influential primarily because of its convincing, logical arguments, but in 1859 Darwin was unable to provide a single empirical case of natural selection. By the late 19th century, two key examples of natural selection became known: mimicry in heliconian butterflies and rapid increases in melanic forms of the peppered moth (*Biston betularia*) as well as of many other moth species in industrial Britain [1,2]. Only now, however, are we beginning to catch a glimpse of the genetics underlying these adaptive changes. Remarkably, two independent and different-looking colour pattern switches in Lepidoptera — one in wing colour patterning and one that melanizes all scales over the wings and body — have been mapped to exactly the same gene in *Heliconius* and *Biston* [3,4].

Mimicry and camouflage have long been a battleground for debates about the nature of adaptive evolution. Mimicry is the matching of the colour pattern of a species that is unpalatable to predators by one that is less-protected, whereas camouflage is in essence mimicry of the local background environment (in the case of melanic *Biston*, this environment is the bark of trees blackened by soot pollution during the Industrial Revolution). In both cases, vulnerable species copy colour patterns seen as inedible to predators.

Early geneticists found that many polymorphisms in nature were inherited as Mendelian loci. For example, Punnett [5] reviewed evidence that a mimicry polymorphism in *Papilio polytes* is inherited at a single locus. This led early Mendelians to argue that melanism and mimicry evolved by mutational leaps rather than the slower, multiple factor, incremental process of natural selection envisaged by Darwin [5–7]. Genetic crosses also showed a single major-effect locus in melanism in peppered moths, but J.B.S. Haldane negated the Mendelians' arguments by demonstrating that the rapid increase of melanic *Biston* in Britain was most likely due to strong natural selection, and in so doing developed perhaps the first ever estimate of the strength of natural selection on a gene locus in nature [8].

Ronald Fisher [9], on the other hand, attacked Punnett's mimicry claims and vigorously defended a more gradualistic Darwinian explanation. He acknowledged that some major phenotypes, for example the phenotype of sex, are controlled at a single switch locus in some species; however, females could not possibly have arisen from males, nor *vice versa*, by a single lucky mutation. Therefore, it is more likely that the single-locus sex switch arose via progressive recruitment of multiple unlinked 'modifier loci' that enhanced and amplified the effects of that locus. By analogy, Fisher argued, a mimicry switch locus should evolve gradually, by recruiting more and more modifiers until its own effect was major [10]. As we shall see, today's empirical findings could hardly have been imagined by either opposing camp.

Early genetic studies had indeed correctly shown that melanism in *Biston* and mimicry phenotypes in some butterflies were inherited as single loci. But the debate about whether mutation or natural selection was chiefly responsible for new phenotypes carried on in ignorance of how any actual 'genes' for melanism or mimicry might specify different colour patterns. The problem was hard to resolve because, until a decade ago, tools to identify changes in DNA responsible for shifts in the wing colours of a lepidopteran were unavailable or prohibitively expensive.

Lepidoptera lack the genetic resources and functional genomic tools of model organisms such as *Drosophila* fruitflies but provide some advantages for evolutionary genetics. In addition to dramatic adaptive phenotypes such as melanism and mimicry, Lepidoptera usually have 20–30 chromosomes, many more than in *Drosophila*, each of which will undergo recombination at every meiosis. They also generally lack inversion polymorphisms that inhibit recombination within chromosomes and make fine-scale mapping of adaptive traits in natural populations of flies difficult. Recombination mapping in controlled lab crosses and association mapping in natural populations, in combination with high-throughput genotyping, therefore can be very efficient in Lepidoptera. Lepidoptera also have relatively compact genomes, at least compared with vertebrates, so that whole-genome resequencing is today a readily applied tool for population studies. In *Biston* and *Heliconius*, this type of classical recombination mapping coupled with advances in sequencing technology and comparative genomics have enabled accurate pinpointing of candidate genomic regions underlying melanism and mimicry [11].

Van't Hof *et al.* [3] recently completed what they dubbed in an interview with the BBC the 'excruciatingly tedious process' of checking all the nucleotide differences, one by one, between melanic and non-melanic *Biston* in just such a candidate region. Reduced polymorphism in the

region for the melanic form complicates recombination mapping but clearly indicates that a rapid selective sweep took place. In fact, a single ~400 kilobase haplotype is still found around this region in approximately half of all melanics; recombination over the past 200 years since its origin has been insufficient to break up the initially favoured haplotype. After exhaustive elimination, only a 22 kilobase transposable element insertion within a large intron of the gene *cortex* correlated perfectly with the melanic phenotype (Figure 1).

The identified gene is a surprising one, as the only previously reported function of *cortex* was in cell-cycle regulation during *Drosophila* meiosis. Nonetheless, the insertion is also associated with up-regulation of one isoform of the gene at a critical period of wing development in the pre-pupal stage of *Biston*. The two intact copies of the novel transposable element within the insertion appear not to be transcribed and are now presumably inactive, and Van't Hof *et al.* [3] therefore conclude that the insertion led to a change in *cortex cis*-regulation leading via a yet unknown mechanism to increased melanization. Melanism in the peppered moth now joins a long list of cases where transposable elements have been exploited by natural or artificial selection during rapid adaptive change, for example, in the evolution of insecticide resistance and the domestication of corn [12,13].

In *Heliconius* butterflies, a similar process of recombination mapping, high-throughput sequencing, and association analysis has led simultaneously to the conclusion that its major mimicry switch locus is located at *cortex* [4]. In *Heliconius*, there are many nucleotide differences associated with the different morphs, but genetic divergence between morphs within *cortex* is much greater than outside the gene. Most DNA divergence is found in the large introns of this gene, and again this is correlated with expression differences of some isoforms of *cortex* in the developing pupal wing. Whereas melanism in the peppered moth likely took place via a single change, multiple changes were almost certainly required to fine-tune complex mimicry patterning across the wings of these butterflies. In one of the species, *H. numata*, an inversion spans *cortex* and several other genes and strongly suppresses recombination, allowing maintenance of a mimetic polymorphism that is rarely broken down. Although expression evidence points mainly to the *cortex* gene itself, the authors do not entirely rule out effects of unidentified non-coding RNAs or *cis*-regulatory effects on other genes in the region. Perhaps the most surprising feature of these discoveries is that the *cortex* region not only acts in *Biston* and *Heliconius*, but also is implicated in the development of colour pattern in other Lepidoptera such as the butterfly *Bicyclus* and the silk moth *Bombyx*.

It now seems clear that Fisher was in detail wrong at least about some major switch loci: *Biston* melanism clearly arose by a single 'hopeful monster' mutation, a transposable element insertion that just happened to give its carrier a major fitness advantage in industrialized Britain. Melanism involves a simple increase in melanin expression over the entire wing and body surface, so perhaps in this case a major-effect mutation was particularly likely. Its success as a phenotype did, however, require strong natural selection to spread a single mutant haplotype to high frequency in industrial regions.

In contrast, we have hints in butterfly mimicry that occasional large-effect inversions may trap multiple sites that give rise to different morphs, but that the evolution of detailed pattern-matching mimicry likely required a more gradual accumulation of multiple changes, contrary to the views of both Punnett and Goldschmidt [5,6]. Nonetheless, we are led to a somewhat modified view of how this gradual change occurred. Whereas Fisher postulated that multiple, unlinked loci were recruited to produce and fine-tune divergent phenotypes triggered by a switch locus, we now begin to understand that many of the nucleotide changes in a mimicry switch are tightly linked and contained within perhaps a single gene and its associated *cis*-regulatory elements.

Similar recent work in *Heliconius* and other mimicry systems seems to bolster this view [11]. For example, a 130 kilobase inversion around the sex-determination gene *doublesex* is the locus of a mimicry switch in *Papilio polytes* [14,15]. In his argument with Punnett, Fisher used the sex-locus as an analogue of a mimicry switch: it seems unlikely that he would have predicted that a sex-switch locus itself could be the same as the mimicry locus. In a bizarre coincidence, the sex-switching *doublesex* gene turns out to be the very mimicry locus in *Papilio polytes* over which Punnett and Fisher argued a century ago [5].

Now the functional work must begin to elucidate precisely how genes as seemingly unlikely as *cortex* or *doublesex* were co-opted into regulating wing colour patterns across the Lepidoptera. And why was *cortex* re-used so often?

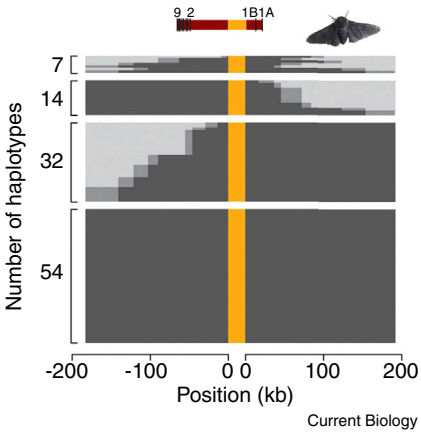

Figure 1. Haplotypes of the melanic form of *Biston betularia* around the *cortex* gene, showing evidence for a rapid selective sweep.

The intron and exon structure of the cortex gene is shown at top (maroon; exons 1A and 1B are alternative first exons; yellow: transposable element insertion). Below, 400 kilobase melanic haplotype sequences near the cortex gene are shown in dark grey where inferred to be identical with the original melanic insertion haplotype, or pale grey if the region results from recombination with ancestral non-melanic haplotypes (intermediate grey represents breakpoint uncertainty). Fifty-four, about half the melanic sequences are unrecombined since the origin of the melanic haplotype in the late 18th or early 19th century. (Reprinted by permission from Macmillan Publishers Ltd: Nature [3], copyright 2016.)

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Vision: Melanopsin and the Pharmacology of Photons

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Using a targeted chemogenetic approach, a new study provides evidence for a unique pathway for neural processing of light information from melanopsin ganglion cells. These results suggest how light can have both alerting and sleep-promoting effects in mice.

While we are most familiar with the retina as the tissue subserving vision — with rod and cone opsins serving as the fundamental photopigments — work over the past two decades has shown that the eye also expresses additional opsin photopigments that support primarily non-visual functions. Melanopsin (Opn4) is expressed in a few thousand retinal ganglion cells (RGCs, the neurons whose axons comprise the optic nerve); its expression renders these cells intrinsically photosensitive. Unlike the majority of RGCs, which project primarily to visual centers of the brain such as the dorsal lateral geniculate nuclei or the superior colliculi, these intrinsically photosensitive melanopsin-expressing RGCs (mRGCs) project primarily to non-visual areas of the brain including the suprachiasmatic nuclei (SCN, locus of the master circadian pacemaker) and the olivary pretectum (integrating nucleus of the pupillary light reflex). Indeed, visually blind mice lacking all rods and cones still demonstrate entrainment of their behavioral circadian rhythms to light–dark cycles and show intact pupillary light responses; but both phenomena are lost in rodless/coneless mice lacking melanopsin [1,2]. However, mRGCs also function as regular ganglion cells, transducing rod and cone signals to their targets even in the absence of melanopsin pigment. This redundant signaling pathway — whereby mRGCs can utilize either 'upstream' rod/cone information or intrinsic melanopsin signaling to communicate with central nervous system targets — has limited efforts to date to understand what function melanopsin may uniquely serve *in vivo*. In a new study reported in this issue of *Current Biology*, Milosavljevic *et al.* [3] utilize a chemogenetic technique to selectively activate melanopsin-expressing mRGCs specifically in order to study their effects on behavior.

The researchers infected one eye of *Opn4*$^{Cre/+}$ mice with an adeno-associated virus (AAV) vector carrying a Flip excision (FLEx)-switched Designer Receptor Exclusively Activated by Designer Drugs (DREADD) receptor, hM3Dq. This receptor has been engineered to activate the G_q signaling G-protein following binding of a synthetic ligand, clozapine N-oxide (CNO). The FLEx domain in the virus ensures that the receptor will only be expressed in the presence of the Cre recombinase, which in turn is driven only in melanopsin-expressing cells (Figure 1). Thus, CNO in theory should mimic the effect of light on mRGCs, as melanopsin is believed to couple primarily to G_q [4]. The group demonstrated that their AAV vector infected about a third of the native mRGCs, and that hM3Dq was expressed only in these cells. The researchers demonstrated that administration of CNO mimicked known light effects on mRGCs, including stimulating pupillary constriction and phase shifting free-running circadian rhythms. Neither effect was seen in the absence of either CNO or AAV infection.

The group then asked the question, what brain regions are activated in response to mRGC cell firing? Induction of the immediate early gene c-fos was used as a surrogate marker for neuronal activation. Not surprisingly, when CNO was given in the early subjective night, the SCN showed marked c-fos induction. Other