Heredity (2012) 108, 592–593

www.nature.com/hdy

Heredity

NEWS AND COMMENTARY

Butterfly wing colours are driven by the evolution of developmental heterochrony

Butterfly wing colours and patterning by numbers

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Heredity (2012) **108,** 592–593; doi:10.1038/hdy.2012.6; published online 29 February 2012

Butterfly wing patterns are rapidly becoming the topic of choice for studies in evolution and development. One of the reasons for the recent rapid 'take-off' in this field is the application of state-of-the-art sequencing and mapping approaches to the elucidation of the genes involved. For several years, only a candidate gene-based approach has been used to look at the redeployment of transcription factors involved in patterning the early embryo in the patterning of the adult butterfly wing. Now, with the advent of the first butterfly genomes (Zhan *et al.*, 2011) and detailed linkage maps (Beldade *et al.*, 2009), we can, for the first time, map colour-pattern genes with no prior assumptions about what they encode. This new approach is elegantly illustrated in a recent study of *Bicyclus anynana* where two melanic mutants (*chocholate*, which affects larvae, and *melanine,* which affects the adult butterfly) have been placed on a gene-based linkage map (Saenko *et al.*, 2012). This study not only illustrates the power of genetic mapping in colour-pattern gene mapping but also reemphasises that 'patterning' and 'pigmentation' are two distinct but clearly linked processes.

If we cast our minds back to simpler times, we can probably remember being given our first colouring book as a child. To simplify and guide the application of colours (akin to pigmentation) to the pre-drawn pictures, these books often contain numbered areas (or pre-determined 'patterns') to guide our early colouring efforts. In a similar way, the developing wing of the butterfly is already 'patterned' long before pigmentation occurs just prior to the butterfly hatching from the pupa. This 'pattern' is not a pattern of colour but a developmental template that alters either the morphology of the wing scales (for example, pigmented versus iridescent scale morphologies) or, critically, the rate at which they develop. The rate of scale development is critical as pigments are only available at fixed time points in development. Thereby, by either slowing or speeding the rates at which scales develop within different pattern elements, they can change colour, be accentuated or even disappear from the wing altogether. This coordinate regulation of scale maturation is a kind of developmental 'heterochrony', where resetting the developmental clock leads to a different final phenotype or colour pattern.

This concept of developmental heterochrony in wing patterning was framed by Bernhard Koch and others 10 years ago (Koch *et al.*, 2000), but unfortunately the importance of the concept has not been widely appreciated by the field. Koch used two examples to illustrate how changes in the rate of scale development drove the final outcomes of pigmentation. The first was the classic example of melanism in the Eastern tiger swallowtail butterfly *Papilio glaucus*, of North America, where melanic females are thought to mimic the distasteful pipevine swallowtail butterfly *Battus philenor*. In this example, scales in the yellow 'background' of the wild-type yellow and black striped female delay their development and become melanised black or brown. Slowing the rate of development of the yellow scales changes their colour as pigments are supplied first to scales destined to be full of yellow pigment (papiliochrome) and then the scales around them are later melanised black to form the tiger stripes of the butterfly (Koch *et al.*, 2000). Thereby, by slowing yellow-scale development, the scales are forced to pigment in the wrong (melanic) time window and end up being a dirty brown colour, presumably enough to fool any potential avian predators!

The second example that Koch chose to study was one from the same butterfly, *B. anynana*, studied by Saenko and others in the paper under current discussion. Rather than choosing another melanic example, he chose to study the *spotty* mutant that carries extra eyespots on the forewing. These 'extra' spots are driven by extra foci of scale development, and therefore, effectively 'acceleration' of scale development rather than the deceleration is seen in the melanic swallowtail. So how does this appreciation of developmental heterochrony and changes in scale development help us to understand a darkened adult butterfly (*melanine*) or a darkened larval cuticle (*chocholate*)? In both these mutants, the overall 'pattern' remains the same but specific pattern elements are darkened. For example, in the *melanine* mutant, the normally buff-coloured ring of the eyespot has been shaded over in brown, in a manner reminiscent of melanisation in *P. glaucus*. Other pattern elements, such as the bands, that surround the eyespots are picked out in darker black. Clearly to see if the buff-coloured scales in the *melanine* eyespots have indeed had their rates of development delayed, thus turning them brown, we would need to examine them at different stages of development under a scanning electron microscope. Unfortunately, such studies were not performed by Saenko and others, however, the authors did manage to presumptively exclude the homologue of the fly pigmentation gene *black* as the *melanine* locus. In fruit flies, the product of the *black* locus (aspartate 1-decarboxylase) is involved in the production of beta-alanine, which is a substrate for the melanin involved in blackening the cuticle. If *melanine* is not a homologue of the fruit fly *black* gene, it may still encode an enzyme responsible for another step in melanin synthesis, thereby effectively giving a 'black' phenotype.

What about the second mutant *chocholate* that darkens only the larval cuticle and appears not to affect the adult? In this case, the authors were able to map the mutant to a specific candidate, a gene encoding the enzyme cysteine sulfinic acid decarboxylase.

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Although the role of this enzyme in larval pigmentation is currently not clear, it is tempting to speculate that this decarboxylase is involved in the complex process of cuticle sclerotisation and that changes in its expression result in a darkened or maybe even hardened cuticle. As newly formed cuticle has to be 'hardened' or 'sclerotised', both the wing scales of the adult wing and the newly deposited cuticle of a freshly moulted larva are exposed to this chemical cascade whose components remain poorly elucidated. So, in conclusion, we can see that it is not just the transcription factor-driven pre-pattern that is important in the final colour of the butterfly wing but that the timing and intensity of pigmentation are driven by the coordinately and finely regulated development of the butterfly wing scales themselves. The regulation of this fine tuning of scale development is poorly understood, but as scale pigmentation is set against a falling titre of ecdysone just prior to butterfly hatching, ecdysone receptors must clearly have a role (Koch *et al.*, 2003). In conclusion, future studies of genetic regulation must pay more attention to the different stages of scale development, pigmentation and maturation. Only by more closely linking these processes will we understand the true role of heterochrony in driving butterfly wing colouration.

CONFLICT OF INTEREST

The author declares no conflict of interest.

Beldade P, Saenko SV, Pul N, Long AD (2009). A gene-based linkage map for *Bicyclus anynana* butterflies allows for a comprehensive analysis of synteny with the lepidopteran reference genome. *PLoS Genet* **5**: e1000366.

Koch PB, Behnecke B, ffrench-Constant RH (2000). The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr Biol* **10**: 591–594.

Koch PB, Lorenz U, Brakefield PM, ffrench-Constant RH (2000). Butterfly wing pattern mutants: developmental heterochrony and co-ordinately regulated phenotypes. *Dev Genes Evol* **210**: 536–544.

Koch PB, Merk R, Reinhardt R, Weber P (2003). Localization of ecdysone receptor protein during colour pattern formation in wings of the butterfly Precis coenia (Lepidoptera: Nymphalidae) and co-expression with Distal-less protein. *Genes Dev Biol* **212**: 571–584.

Saenko SV, Jerónimo MA, Beldade P (2012). Genetic basis of stage-specific melanism: a putative new role for a cysteine sulfinic acid decarboxylase in insect pigmentation. *Heredity* **108**: 594–601.

Zhan S, Merlin C, Boore JL, Reppert SM (2011). The monarch butterfly genome yields insights into long-distance migration. *Cell* **147**: 1171–1185.

Editor's suggested reading

Beldade P, Brakefield PM (2002). The genetics and evo-devo of butterfly wing patterns. *Nat Rev Genet* **3**: 442–452.

Beldade P, Mateus ARA, Keller RA (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Mol Ecol* **20**: 1347–1363.