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NEWS AND COMMENTARY

Carotenoid genetics

Chicken skin sheds light on carotenoid genetics

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The conspicuous yellow or red carotenoid colouration found in many birds is surprisingly poorly understood from a genetic standpoint. Many studies have established carotenoid colouration as an honest indicator of male quality (Blount *et al*., 2003; Faivre *et al*., 2003; Blas *et al*., 2006) but the genetic mechanisms involved in metabolizing and depositing the pigments have remained elusive. This is unfortunate as genes controlling these processes could be potential keys to unlocking how sexual selection acts at a molecular level. A recent paper by Eriksson *et al*. (2008) has elucidated the genetic basis for the carotenoid trait in chickens and uncovered a mechanism for carotenoid allocation that is quite the reverse of what many would have expected.

The trait studied was the presence of yellow legs—a common phenotype in domestic chickens. It was previously known that this is a Mendelian trait that maps to chromosome 24, with the yellow skin allele (*W*Y*) being recessive (Schmid *et al*., 2000). Eriksson *et al*. (2008) used a combination of identical-by-descent mapping and linkage analysis on a yellow skin × white skin backcross to refine linkage to a 23.8 kb region that contains only two genes: *BX935617* and *BCDO2*. Resequencing of this region revealed a large 0.8% sequence divergence (over 200 nt) between the two haplotypes, making identification of causative mutation(s) difficult. Gene expression analysis (reverse transcription PCR) on white and yellow skin tissue showed only a weak expression of *BX935617*. However, reverse transcription PCR and pyrosequencing of *BCDO2* from skin of six heterozygous birds showed moderate expression and, notably, that 90% of the transcripts were from the white skin (*W*W*) allele. This contrasts strongly with *BCDO2* expression in the liver, which showed more equal representation of the two alleles. *BCDO2* encodes an enzyme, β-carotene dioxygenase-2 (or β-carotene-9′,10′-monooxygenase), that asymmetrically cleaves β-carotene to create colourless apocarotenoids (Kiefer *et al*., 2001).

The authors therefore propose that the yellow skin phenotype is determined by one or more tissue-specific *cis*-acting regulatory mutations affecting *BCDO2* expression. When the *W*Y* allele of *BCDO2* is homozygous, its expression is low in skin tissue, the carotenoid remains intact and is incorporated into keratinocytes. In white-legged chickens, carotenoid deposition in the skin is prevented at the final hurdle by the expression of a cleaving enzyme in the skin tissue itself. An obvious test would be to measure the expression of *BCDO2* in homozygous *W*Y/W*Y* birds—if the proposed mechanism is correct, then reduced expression of *BCDO2* would be expected. It will also be necessary to demonstrate that BCDO2 is able to cleave lutein and/or zeaxanthin, which are the carotenoids deposited in chicken skin. A mutation affecting skin colour has previously been identified—the white-legged Wisconsin hypoalpha mutant (WHAM) chicken has a loss-of-function mutation in *ABCA1* (Attie *et al*., 2002). In this case, white skin is one of a suite of traits affected, and the loss of carotenoid is probably related secondarily to a severe disruption in lipid transport, which prevents the delivery of carotenoids around the body. In contrast, the yellow skin phenotype affects only skin colouration, making this the first example of identification of the genetic basis of a specific carotenoid-based colour trait in any vertebrate.

The study also casts revealing light on the origin of the domestic chicken. Since Darwin (1868) proposed that domestic chickens were derived solely from red junglefowl (*Gallus gallus*) there has been much debate about a potential contribution from other junglefowl species in South Asia, and the geographical location(s) of domestication is still unresolved (Hutt, 1949, Liu *et al*., 2006). Eriksson *et al*. (2008) resequenced the 23.8 kb region from all four junglefowl species and found that *W*Y* haplotypes from yellow-skinned domestic chicken cluster into a single clade containing two wild species, the grey junglefowl, *G. sonneratii*, and the Ceylon junglefowl, *G. lafayettii*, which have reddish and yellow legs, respectively. In contrast, *W*W* haplotypes group within a clade containing sequences from the red junglefowl, which has white legs. This is in striking contrast to other loci such as mtDNA where all haplotypes from domestic chicken cluster with red junglefowl. Thus the *W*W* and *W*Y* alleles segregating in domestic chickens have independent origins from wild birds, and the most likely origin for the *W*Y* allele is through hybridization with the grey junglefowl during domestication. As the authors suggest, it would be interesting to perform a larger genomic study to see whether introgression from other species is a more widespread phenomenon. From an evolutionary perspective, it is notable that although the study initially focused on a mutation in domestic chickens, both the phenotypic and genetic variations underlying the trait are found among wild species, specifically the red junglefowl of South East Asia and the grey junglefowl of India; so this study is hugely relevant to the evolution of carotenoid-based colouration in nature.

Many showy carotenoid displays are used to attract mates or signal to members of the same sex, and there has been much debate as to how these displays are able to be honest indicators of quality. It is of considerable importance to know if carotenoid traits are genetically correlated with other traits that may confer a fitness advantage. It is therefore interesting that Eriksson *et al*. (2008) tested for association between yellow skin and a suite of 80 other traits (for example, growth, egg production, behaviour) in a quantitative trait loci analysis. However, no evidence of a genetic correlation was found—after correction for multiple tests, no traits were significantly associated with yellow skin genotype. Of course, this does not address whether there are correlations between carotenoid traits and other traits in wild populations, but it does show that carotenoid variation is not necessarily associated with strong pleiotropy.

The real surprise of the study is the nature of the probable mechanism uncovered. Although not emphasized by the authors, the results strongly suggest that the ancestral state in junglefowl is yellow skin. This follows as the white

skin phenotype is due to a gain of function—an increase in expression of *BCDO2* in the skin promoting carotenoid cleavage, and no other function for *BCDO2* in the skin has been proposed. The carotenoid uptake through the gut, metabolism and storage in the liver and circulation in the blood appear to be unaffected—it is only the final deposition stage. This goes against the prevailing view that carotenoid-based traits in birds are generally derived. This study therefore leads to many novel avenues of research. Is this a mechanism for variation in carotenoid colouration among other species? Does this enzyme play a role within an individual to limit carotenoid deposition to certain areas of the body? These are questions that deserve extensive investigation. For carotenoid researchers, perhaps future emphasis should not only be placed on why some birds are colourful, but also on why others are not.

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Attie AD, Hamon Y, Brooks-Wilson AR, Gray-Keller MP, MacDonald MLE, Rigot V *et al.* (2002). Identification and functional analysis of a naturally occurring E89K mutation in the ABCA1 gene of the WHAM chicken. *J Lip Res* **43**: 1610–1616.

Blas J, Perez-Rodriguez L, Bartolotti GR, Vineula J, Marchant TA (2006). Testosterone increases bioavailability of carotenoids: Insights into the honesty of sexual signalling. *Proc Natl Acad Sci USA* **49**: 18633–18637.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF (2003). Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**: 125–127.

Darwin C (1868). *The Variation of Animals and Plants Under Domestication*. John Murray: London.

Eriksson J, Larson G, Gunnarsson U, Bed'hom B, Tixier-Boichard M, Strömstedt L *et al.* (2008). Identification of the *yellow skin* gene reveals a hybrid origin of the domestic chicken. *PLOS Genet* **4**: e1000010.

Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G (2003). Immune activation rapidly mirrored in a secondary sexual trait. *Science* **300**: 103.

Hutt FB (1949). *Genetics of the Fowl*. McGraw Hill Book Company Inc: New York.

Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE *et al.* (2001). Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. *J Biol Chem* **276**: 14110–14116.

Liu YP, Wu GS, Yao YG, Miao YW, Luikart G, Baig M *et al.* (2006). Multiple maternal origins of chickens: out of the Asian jungles. *Mol Phylogenet Evol* **38**: 12–19.

Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn M, Buerstedde JM *et al.* (2000). First report on chicken genes and chromosomes 2000. *Cytogenet Cell Genet* **90**: 169–218.

Editor's suggested reading

Fairbairn DJ, Roff DA (2006). The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. *Heredity* **97**: 319–328.

Hoekstra HE (2006). Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity* **97**: 222–234.

Joron M, Jiggins CD, Papanicolaou A, McMillan WO (2006). *Heliconius* wing patterns: an evo-devo model for understanding phenotypic diversity. *Heredity* **97**: 157–167.

Parichy DM (2006). Evolution of danio pigment pattern development. *Heredity* **97**: 200–210.