



PERSPECTIVES

EVOLUTION

How fish get their stripes—again and again

Cichlid fish emerge as new models for pattern development and evolution

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Conspicuous or otherwise elaborate color patterns sported by fishes, which constitute half of all living vertebrates, are among the most fascinating, varied, and intricate of nature. The importance of coloration for fishes can be appreciated from its many roles in camouflage and communication. The molecular and cellular mechanisms underlying color pattern development and evolution in teleost fish have received considerable attention, mostly through work in the zebrafish, *Danio rerio*, and its relatives. In these

species, adult horizontal stripes, vertical bars, and spots along the body are formed by the timely expression of relevant genes and the orderly migration and interactions between three cell types originating from embryonic neural crest precursors: dark melanophores, yellow and orange xanthophores, and iridescent iridophores. These cells migrate to their final destination in the hypodermis and interact with each other at short and long ranges, and with their tissue environment, to produce color patterns predicted by Turing reaction–diffusion models, in which periodic patterns are produced by the interaction of an activator and an inhibitor (1–6). On page 457 of this issue, Kratochwil *et al.* (7) show that the degree of expression of the newly identified color pattern gene, *agouti*-related

peptide 2 (*agrp2*), in the skin acts as a molecular switch mechanism controlling the presence or absence of horizontal melanic stripes in African cichlid fish.

Cichlids from the East African Great Lakes—Victoria, Tanganyika, and Malawi—have the highest sustained rates of speciation among vertebrates. Therefore, they offer perhaps the most diverse array of phenotypes for studying color pattern development and evolution in vertebrates. Kratochwil *et al.* used a combination of crosses between striped and nonstriped species pairs, fine-mapping of F₂ hybrid offspring and populations, gene expression assays, and functional experiments to narrow down the causative region to a 1.1-kb portion of the first intron that cis regulates expression of *agrp2*. These data indicate that

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higher expression of *agrp2* represses horizontal melanic stripes in African cichlids.

Antimelanogenic *Agouti* genes have been implicated in color patterning in tetrapods through embryonic expression and establishment of a prepattern (8, 9). Although *agrp2* expression differences in the skin of striped and nonstriped cichlids have been identified in adults, there are no obvious local differences that could explain the coordinates of stripe location. Indeed, *agrp2* expression levels are similar in melanic and nonmelanic skin (7). Thus, it is possible that an embryonic prepattern mechanism, conserved across vertebrates and involving any of the *Agouti* ohnologs (genes that arise from whole-genome duplication), also exists in cichlids. Alternatively, melanophore migration and patterning might be following particular anatomical landmarks, defining a boundary and followed by more localized interactions with other pigment cell types and the extracellular environment, to shape stripes in their correct location, possibly following Turing principles. Interestingly, melanic bars seem unaffected by the expression patterns of *agrp2* in the

adult skin. This suggests the existence of different melanophore lineages, each with distinct bar or stripe fates, such as melanophores of embryonic or postembryonic origin in *Danio* species (10). Ablation of *agrp2* expression with CRISPR-Cas9 reconstitutes a midlateral stripe in a nonstriped cichlid but not a dorsolateral stripe. This points to additional genetic or cellular factors contributing to stripe phenotypes in cichlids.

Owing to their high species and phenotypic diversity, yet close phylogenetic relationship and availability of genome resources (11), the cichlids enable testing for molecular mechanisms involved in parallel or convergent evolution like no other vertebrate model. Stripes are lost and gained multiple times according to high or low expression of *agrp2* in the skin in different cichlid radiations from Lakes Victoria, Tanganyika, and Malawi (7). Interestingly, regulation of *agrp2* expression is achieved by different, rather than the same, genetic polymorphisms. These findings highlight how cis regulation might be a primary mechanism for generating phenotypic diversity from the same genes without requiring

Re-evolution (gain and loss) of horizontal stripes in cichlid fish from Lakes Victoria, Tanganyika, and Malawi is controlled by labile regulatory switches of *agrp2* expression.

functional differences in protein sequence, particularly if those genes have a cascading effect on phenotype based on timing and location of their expression (2, 5, 8, 12, 13). Hybridization and recombination-mediated reshuffling of developmentally important cis-regulatory regions is a plausible mechanism to achieve high phenotypic diversity in a short period of time, as in cichlids from Lakes Victoria and Malawi, especially given the unexpectedly low mutation rates (14, 15).

Much of the variation in the thousands of cichlid species is in the form of exacerbated color patterns and craniofacial phenotypes, which originate from a vertebrate evolutionary innovation—the neural crest. It seems that cichlids have harnessed this embryonic tissue to become one of the most diverse groups of vertebrates alive, and we now have better tools to investigate that. The study of Kratochwil *et al.* illustrates how advances in genetic engineering, driven by CRISPR-Cas9, are bringing classical models in ecology, evolution, and behavior to the molecular developmental biology laboratory. A new era has begun in which it is possible to demonstrate not only how cichlids get their stripes and how that might differ from mechanisms shaping zebrafish and tetrapod stripes but also how the seemingly independent stripe and bar modules of cichlids are regulated and how cell-cell interactions are coordinated during color pattern formation. It will finally be possible to answer long-standing questions about how the astonishing phenotypes of cichlids (head, body, and fin coloration; craniofacial shape; oral and pharyngeal dentition; fin shape; social and reproductive behaviors; breeding systems; sex, and even extended phenotypes like mating structures) are produced and often replicated throughout evolutionary history. ■

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