

## NEWS AND VIEWS

## PERSPECTIVE

## The shape of things to come in the study of the origin of species?

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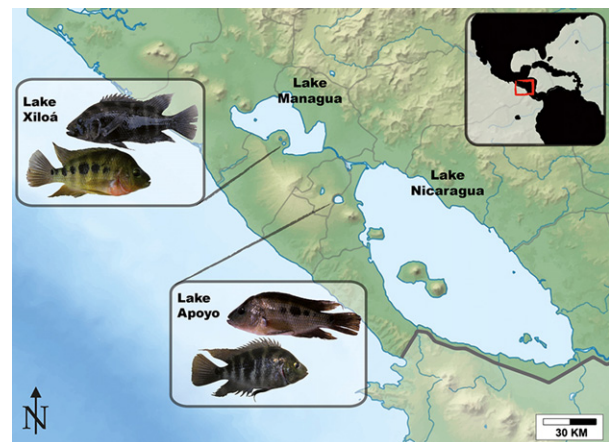
Perhaps Darwin would agree that speciation is no longer the mystery of mysteries that it used to be. It is now generally accepted that evolution by natural selection can contribute to ecological adaptation, resulting in the evolution of reproductive barriers and, hence, to the evolution of new species (Schluter & Conte 2009; Meyer 2011; Nosil 2012). From genes that encode silencing proteins that cause infertility in hybrid mice (Mihola *et al.* 2009), to segregation distorters linked to speciation in fruit flies (Phadnis & Orr 2009), or pollinator-mediated selection on flower colour alleles driving reinforcement in Texan wildflowers (Hopkins & Rausher 2012), characterization of the genes that drive speciation is providing clues to the origin of species (Nosil & Schluter 2011). It is becoming apparent that, while recent work continues to overturn historical ideas about sympatric speciation (e.g. Barluenga *et al.* 2006), ecological circumstances strongly influence patterns of genomic divergence, and ultimately the establishment of reproductive isolation when gene flow is present (Elmer & Meyer 2011). Less clear, however, are the genetic mechanisms that cause speciation, particularly when ongoing gene flow is occurring. Now, in this issue, Franchini *et al.* (2014) employ a classic genetic mapping approach augmented with new genomic tools to elucidate the genomic architecture of ecologically divergent body shapes in a pair of sympatric crater lake cichlid fishes. From over 450 segregating SNPs in an F2 cross, 72 SNPs were linked to 11 QTL associated with external morphology measured by means of traditional and geometric morphometrics. Annotation of two highly supported QTL further pointed to genes that might contribute to ecological divergence in body shape in Midas cichlids, overall supporting the hypothesis that genomic regions of large phenotypic effect may be contributing to early-stage divergence in Midas cichlids.

**Keywords:** adaptation, covariation, ecological genomics, geometric morphometrics, linkage map, phenotypes, QTL, shape, speciation

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Cichlid species flocks are a prime example of recent adaptive radiations. The Midas cichlid species complex (*Amphilophus* spp.) of Nicaragua provides one of the few known examples of sympatric speciation where species have rapidly evolved parallel morphologies in young crater lakes (Fig. 1). Adaptive radiations of the *Amphilophus* species complex include remarkable divergence in colour, lip morphology and pharyngeal jaw morphology (Meyer 2011). Body shape divergence along the deep-elongated axis of variation ('elongated' versus 'high-bodied', i.e. limnetic and benthic) has repeatedly evolved in the presence of gene flow. Repeated evolution of similar phenotypes suggests a role for natural selection in the evolution of reproductive isolation (McGee & Wainwright 2013).

Remarkably, cichlid radiations in body shape mirror the evolutionary trajectories of other fish species complexes (e.g. stickleback and whitefishes) in association with freshwater lake habitats (e.g. limnetic and benthic, Rogers *et al.* 2013). Predation and diet are predicted to be the selective forces driving body shape divergence, and once the ecological annotation of the genes underlying body shape has been confirmed (e.g. (Rogers *et al.* 2013; Pavey *et al.* 2012), further studies can elucidate the fitness consequences of these genes and determine whether divergent selection reduces gene flow (Barrett *et al.* 2008), towards confirming their role in the ecological speciation of the species flocks.



**Fig. 1** Two crater lakes in Nicaragua illustrating the parallel changes in body shape between *Amphilophus astorquii* and *Amphilophus zaliosus*. Lake Apoyo is where the focal species of the QTL work in Franchini *et al.* (2014) was conducted.

Franchini *et al.* crossed a limnetic and benthic representative of the *Amphilophus* complex to generate a high density F2 mapping cross. They quantified phenotypes using geometric morphometrics and elucidated the genetic architecture of body shape. They discovered 11 QTL associated with these ecologically divergent phenotypes. A closer look at the most strongly supported body shape QTL spanning 3 million base pairs against the Tilapia genome revealed 20 annotated genes, with initial observations suggesting two genes that may be associated with bone development and/or metabolism. The mutation(s) associated with these genes, whether they are adaptive, or actually cause reproductive isolation, remains to be determined, but this study demonstrates that molecular ecologists can increasingly integrate this approach as a key step to address this question and other questions surrounding speciation genetics when crosses are feasible.

Perhaps more importantly, Franchini *et al.* highlight the importance of considering ecologically relevant phenotype-environment associations. The tools of geometric morphometrics allow detailed assessments of phenotype, which will increasingly shed light on the genetics of adaptation and the origin of species. Biological shapes are ideal targets for such investigations, because they represent a suite of adaptive traits evolving together that can be measured in the same units (Rogers *et al.* 2012). For this reason, shape traits are also considered ideal by evo-devo enthusiasts to elucidate the role of modularity in facilitating or constraining ecological divergence (Parsons & Albertson 2013; Jamniczky *et al.* 2014).

What can the geometric morphometric quantification of phenotypic variation contribute to speciation genetics? As Franchini *et al.* demonstrate, candidate genes underlying shape variation may be associated with the expression of proteins that modify shape, directly or indirectly. This exemplifies the notion that organisms are integrated to function as a whole, and such functional integration often leads to covariation among quantitative phenotypic traits (Klingenberg 2009). Developmental architecture structures phenotypic variation by producing covariation and modular organization within the phenotype (Hendrikse *et al.* 2007). Natural selection can act on developmental architecture to produce correlation among functionally related traits (Cheverud 1996). While the tendency for developmental processes to produce functional integration can bias the degree and direction of evolutionary change (Wagner *et al.* 1997; Klingenberg *et al.* 2012), covariation structure can be modified by altering the relative variance of different covariance-generating processes without affecting the underlying developmental interactions themselves, thus reducing the degree to which such interactions constrain evolution (Jamniczky & Hallgrímsson 2009; Sanger *et al.* 2011). Indeed, the correlated response to selection produced by development in structuring complex systems may act to enhance the evolvability of such systems (Wagner & Altenberg 1996; Hansen & Houle 2008). Elucidation of the genes involved and their interactions is a key step in understanding the complex relationship between genotype

and phenotype in changing environmental contexts (Stern 2013).

Exploring the nature of processes underlying phenotypic modularity allows us to gain new insight into the means by which selection may produce novel phenotypes over time. Does modular organization constrain or enhance an organism's ability to adapt to a new environment? We can consider the relationship of modularity to evolutionary change from two perspectives: a hypothesis of modular stasis, from which we predict that patterns of modularity are conserved across lineages and constrain the area of phenotype space available for exploration; or alternately, a hypothesis of modular reorganization, from which we predict that patterns of modularity themselves evolve as organisms adapt to new niches. Parsons *et al.* (2012) recently demonstrated that patterns of modularity in the trophic apparatus are conserved within cichlid genera, but have diverged in association with changes in feeding behaviour. In contrast, Jamniczky *et al.* (2014) have demonstrated that patterns of modularity in the trophic apparatus of threespine stickleback are conserved despite similar substantial divergence in feeding behaviour. These results hint at the presence of a complex relationship between development, genetics and evolution in rapidly evolving organisms, and at the possibility that the interplay between genetic and developmental factors can result in different solutions to similar ecological challenges. As Franchini *et al.* demonstrate, the integration of genetic mapping can help elucidate the key candidate genes may be involved in this process. The degree to which covariation structure aligns with the contours of the adaptive landscape through which a population is diverging may influence the rapidity with which evolutionary change can occur (Jamniczky *et al.* 2014). Integration of these aspects with knowledge of genetic architecture of shape, as Franchini *et al.* have performed, promises to shed light into these questions and the role of shape (co)variation and modularity in speciation.

Identification of the number, size and distribution of genomic regions underlying the evolution of divergent adaptive phenotypes represents an important first step in the analysis of the genomic basis of adaptive divergence, the first step in a potentially very long road towards identification of the mutation(s) underlying adaptive phenotypes (Linnen *et al.* 2013). Consequently, such studies will benefit from the application of multiple approaches (genomic scans, analyses of gene expression and QTL studies) on the same sets of populations to better understand the link between phenotype and genomic islands of divergence (Hendry 2013). Such integration of experimental approaches will eventually benefit from also considering additional aspects of experimental design that can hone in on the genes and their role in speciation. For example, replicate genetic crosses stemming from different environmental trajectories or time points in the process of speciation may help elucidate the genetics of adaptive peak shift (Rogers *et al.* 2012). The information garnered from a single cross in Franchini *et al.* highlights the benefits to gain from these considerations.

All that is gold certainly does not glitter in forward genetic approaches (Rockman 2012; Travisano & Shaw 2013), but these recent advances in the Midas cichlid system suggest that these approaches may strike evolutionary gold after all. Indeed, such methods are increasingly promising to elucidate candidate genes underlying phenotypic variation (Barrett & Hoekstra 2011; Baud *et al.* 2013; Parsons & Albertson 2013; Stern 2013), and when combined with integrative experimental approaches, the role of adaptation in speciation may now be within reach. Yet, in addition to integrating these different experimental approaches, molecular ecologists need to keep in mind that the variation selection sees will ultimately be the phenotype (Travisano & Shaw 2013), and approaches that provide more unbiased holistic phenotypic estimates of variation should yield better insights into the process. The integration of geometric morphometric techniques appears to be a step in the right direction. Combined with the power and promise of population genomics, longstanding questions and predictions regarding the nature of species can finally be tested.

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