

Cichlid genomics and phenotypic diversity in a comparative context

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Synopsis Cichlid fishes provide an excellent natural system for integrating studies of genomics and adaptive radiation. Cichlids are unique in comprising a substantial fraction of all vertebrate species, possessing unique jaw structures, displaying an exceptional range of breeding systems, and exhibiting rampant phenotypic convergence. The rate of divergence in cichlid jaws, teeth, color patterns, visual systems, reproductive biology, and mating behaviors is unparalleled among vertebrates. I discuss ways rapid divergence in cichlids and other adaptive radiations make understanding the genomic basis of adaptive divergence more tractable. Then, I briefly overview some major findings and insights into vertebrate adaptation that have been gained through cichlid genetic studies. Finally, I discuss the extensive evolutionary replication provided by cichlid adaptive radiations and their potential for studies of genotype-to-phenotype mapping.

Introduction

Adaptive radiations and genomes represent two disparate levels of biological organization. Meshing the two stretches the limits of integrative biology. The study of adaptive radiation examines the evolution of ecological and phenotypic diversity within a rapidly multiplying group of species (Schluter 2000). Genomics generally involves the characterization of the complete genetic makeup of a single organism including how this information is arranged into genes and encoded into proteins (Lynch 2007). Genome sequencing will not only increase the scale at which adaptive change at the molecular level can be investigated, but facilitate comparisons of genome-wide sequence patterns among rapidly radiating species. Assessing how genetic structure changes during rapid diversification could give an exceptionally lucid view into adaptive evolution because of the increased tractability of isolating adaptive genetic changes in the genome. Also, studying the genomics of adaptive radiations could clarify how the genetic makeup of an organism influences the evolutionary rate of phenotypic and ecological diversification. The soon-to-be-completed sequencing of the genome of several cichlid fishes provides an unprecedented opportunity to study the genomics of adaptive diversification.

To understand the value of cichlids as a model for vertebrate genomics, I first examine the traits that distinguish these fish from all other vertebrates.

Then, I detail the rate that cichlid radiations have evolved and how these rapid accumulations of diversity could provide excellent systems for examining the genetic basis of adaptive evolutionary change. I also describe several phenotypes that have been studied extensively in cichlids using genetic tools to examine how genome structure, molecular evolution, and developmental genetics may broaden our understanding of adaptive evolution. Finally, I discuss how the repeated phenotypic convergence among cichlid radiations should provide us with enhanced insight into the evolutionary processes responsible for adaptive divergence.

What makes cichlids unique

The enormous number of cichlid species makes understanding the genome of these fish critical for an understanding of vertebrate diversity. With estimates of approximately 2500 species of cichlids (Genner et al. 2004), about one out of every 25 vertebrate species on earth is a cichlid. Despite small radiations of cichlids in Madagascar (approximately 30 species) and India (approximately 3 species) most cichlids are concentrated in the Neotropics and in Africa (Chakrabarty 2004). There are approximately 400 species in South America and about 100 species in Central America that together form a monophyletic sister group to the approximately 2000 species found in Africa (Sparks and Smith 2005). The cichlids of Africa also form a

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monophyletic group, and they are most species-rich in the East African Rift lakes (Salzburger 2009). Lake Tanganyika, Lake Victoria, and Lake Malawi, the three physically largest of these rift lakes, harbor the most cichlid species (Genner et al. 2004). Their species richness simultaneously makes cichlids unique and also makes understanding cichlid biology fundamental to understanding the breadth of vertebrate diversity.

The number of cichlids in communities also spans several orders of magnitude (Kornfield and Smith 2000). Low-diversity cichlid communities that contain fewer than 10 species are most often found at the northern and southern edges of the group's distribution. In subtropical lakes and rivers in places like the southern border of the USA or in Israel or Iran, species such as the Texas cichlid or isolated lineages of *Tilapia* are the only cichlids found (Chakrabarty 2004). However, in tropical cichlid communities and especially in the African Great Lakes, cichlid assemblages can include well over 10 and up to 100 sympatric species. In the most species-rich cichlid communities, the stratified habitats surrounding islands found in Lake Malawi can harbor a hundred or more interacting cichlid species (Snoeks and Konings 2004). No other radiation of vertebrates provides such a phenomenal range of species numbers that spans several orders of magnitude among assemblages.

One reason that cichlids can co-exist in such species-rich communities is the extent that species are trophically specialized to exploit particular prey (Greenwood 1964; Fryer and Iles 1972; Bolnick et al. 2003). The mechanistic basis for much of this narrow niche breadth can be traced to the jaws of cichlids (Liem 1973, 1979). Cichlids, like most groups of fish, have two sets of jaws. However, cichlids possess three unusual modifications of their pharyngeal jaws compared to all other groups of fish (Liem 1973). Cichlids have novel insertions of the levator externus IV and levator posterior onto the lower pharyngeal jaw that in most teleosts insert onto the upper pharyngeal jaw. The upper pharyngeal jaws also exhibit novel synovial joints between the neurocranium and the upper pharyngeal jaws. Finally, cichlids have a fused lower pharyngeal jaw that is sutured together and links the pharyngeal muscles into a muscular sling. In other groups, like labrids and damselfish, that possess this pharyngeal sling, the lower pharyngeal jaw is not sutured and forms a single bone (Liem 1973; Stiassny and Jensen 1987). The suturing in the cichlid pharyngeal jaw putatively permits rapid change in response to the mechanical demands of processing particular prey

types (Hulsey 2006). Because of the inherent lability in the robustness of their pharyngeal jaw, cichlids can easily evolve in ways that exploit large prey items, like fish, as well as durable prey items like hard-shelled mollusks (Hulsey et al. 2008).

The innovations in the cichlid trophic apparatus might also have allowed the functional abilities of the oral and pharyngeal jaws to evolve in a modular fashion (Liem 1973; Hulsey et al. 2006). The influence of this modularity on trophic evolution is perhaps best reflected in cichlid species that are polymorphic in one jaw and not the other. Species such as *Herichthys minckleyi* are polymorphic in their pharyngeal jaws with one morphotype adapted to crushing snails while another morphotype is adapted to shredding plants (Hulsey et al. 2005a). This variation appears unrelated to variation in the oral jaws (Hulsey et al. 2006). Other cichlids exhibit polymorphic oral jaws that have diverged independently of variation in the pharyngeal jaws. Cichlid species, like *Perissodus microlepis* (a scale-eater from Lake Tanganyika), have both right- and left-handed morphotypes that specialize in scraping off scales of the left or right side of other cichlids (Hori 1993). These polymorphic species highlight the modularity of cichlid jaws as well as the rate and extent of phenotypic specialization that characterizes cichlid trophic evolution.

Cichlid mouths are also critical components of their reproductive system and sexual selection, and have played a significant role in their extraordinary speciation and phenotypic diversification. In many East African cichlids, including most cichlids in Lakes Victoria and Malawi (Snoeks and Konings 2004), females mouthbrood the young. During mouth brooding, one of the parents raises the young in its mouth from hatching through the early stages of development (Sturmbauer and Meyer 1993). Unlike most mouthbrooding fish groups like apogonid cardinalfish or ariid catfishes in which the male broods the young, the female is almost always the caretaker in mouthbrooding cichlids (Duponchelle et al. 2008). However, cichlid females, even in non-mouthbrooding species, often guard the young (Sturmbauer and Meyer 1993). This type of extensive female investment in offspring is likely one of the primary reasons why female choice is generally strong in cichlids. The intense sexual selection in this group, linked to the disparities between females and males in their contribution to raising offspring, has likely fueled the extraordinary diversity of color patterns, courtship behavior, and the co-evolved visual systems of

cichlids (Carleton et al. 2005; Gerrard and Meyer 2007; Dijkstra et al. 2008).

The repeated convergent evolution among cichlid radiations offers some of the most compelling evidence that particular phenotypes are adaptive. Analogous to the repeated benthic and limnetic morphotypes found in groups such as sticklebacks (Schluter 2000; Boughman et al. 2005) and the habitat-specific ecomorphs of *Anolis* lizards (Losos et al. 1998), cichlids have repeatedly evolved strikingly similar phenotypes that match particular ecological circumstances. Perhaps like no other group of vertebrates, cichlids exhibit extensive convergence in trophic morphology, color patterns, and behavior (Fryer and Iles 1972; Kocher et al. 1995; Ruber et al. 1999; Koblmüller et al. 2004; Hulsey et al. 2008). Critical to mechanistic studies of adaptation, these replicated similarities provide evolutionarily independent lineages that could be used to test the genetic, developmental, and phenotypic basis of ecological specialization.

The appeal of cichlids is not limited to ecological, behavioral, and evolutionary studies. Cichlids are also a model system in physiological studies of ion exchange and homeostasis (Rengmark et al. 2007; Inokuchi et al. 2009). Furthermore, cichlids like *Tilapia* have long been a source of protein in Africa and have become one of the most intensively produced aquaculture species in the world (Coward and Little 2001). Their applied value as a food source is one of the fundamental reasons why cichlid genomes are being sequenced.

Adaptive radiations and the genetic basis of phenotypes

Although cichlids are highly diverse in the Neotropics and other parts of Africa, they reach their pinnacle of rapid phenotypic diversification in the East African Great Lakes (Genner et al. 2004). Several of the East African Cichlid radiations are thought to represent the most rapidly diversifying group of vertebrate lineages (Danley and Kocher 2001). These inferences were first made based on the limited amount of divergence in mitochondrial DNA sequences in these clades (Meyer et al. 1990; Kocher et al. 1995; Verheyen et al. 2003; Salzburger et al. 2005). More recent analyses from the nuclear genome have begun to corroborate that these lineages diversified over very short spans of evolutionary time (Won et al. 2006; Genner et al. 2007). As our understanding of historical biogeography and the fossil record of cichlids becomes more robust, it will be feasible to more rigorously test hypotheses

concerning the timescale of divergence within cichlid radiations (Hulsey et al. 2004; Sparks and Smith 2005; Genner et al. 2007). Understanding how rapidly cichlid radiations have diversified is critical for deciding how best to utilize these fish in genomics research.

Isolating the genetic basis of phenotypic differences is easier in rapidly diversifying adaptive radiations for at least two reasons. First, the greater amount of divergence facilitates identifying and measuring significant phenotypic differentiation. If there is more divergence in the length of a jaw or the intensity of a color pattern, quantifying those differences is less difficult. Second, there is less fixed genetic “noise” between recently diverged species (Fig. 1). Identifying the genetic changes responsible for differences in a phenotype within the vast genome of a vertebrate is like isolating a needle in a haystack containing several billion base pairs. Because mutations steadily accumulate through time via genetic drift in an almost clock-like fashion, species diverging over longer periods have more genomic differences (Fitch and Langley 1976; Lynch 2007). This makes finding mutations responsible for phenotypic differences more difficult (Fig. 1). In many cases, species separated for longer than a few million years have so many fixed differences that it is impossible to determine, which genetic changes are responsible for trait divergence. Alternatively, adaptively radiating species have a much smaller haystack of fixed mutations to sort through in order to find the figurative needle responsible for their substantial phenotypic differences.

Much research into adaptive radiations centers around the number of species in a radiation (Schluter 2000) and the number of cichlid species is extensively debated (Genner et al. 2004; Snoeks and Konings 2004; Salzburger 2009). The exact number of species of reproductively isolated lineages within a given cichlid radiation is not critical to genomics research. However, the high number of cichlid species recognized advantageously reflects the large amounts of phenotypic divergence in these clades. Furthermore, evaluating whether a clade has diversified exceptionally rapidly and/or exhibits unusual amounts of phenotypic divergence can be estimated independently of the number of species and is inherently a comparative question (Collar et al. 2005). One contrast that illustrates this and contextualizes the extraordinary rates of cichlid phenotypic evolution is the differences in diversification between clades of “phenotypically” cryptic species and cichlid radiations that often contain “genetically” cryptic species.

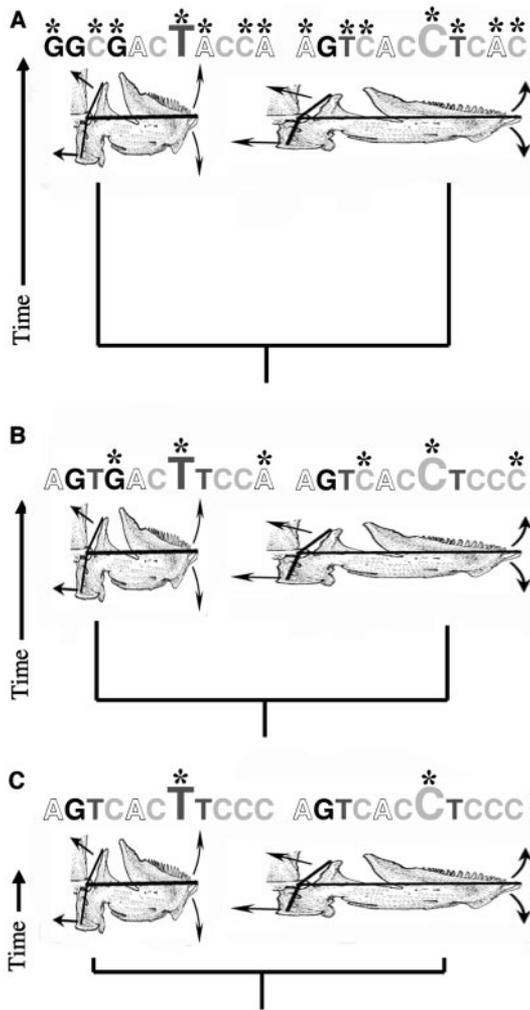


Fig. 1 Determining the genetic basis for phenotypic differences like the size or mechanics of the cichlid lower jaw becomes more difficult the longer two species have diverged. To illustrate this, the DNA sequences of two divergent alleles fixed in two species are shown at three different time-scales (**A**, **B**, and **C**). Like all DNA sequence, these sequences are composed of four bases: adenine (A), thymine (T), guanine (G), and cytosine (C). The bases are shaded different colors to enhance the contrast between them. The locus for each of the two species is depicted and the associated differences in the phenotype of the lower jaw are shown above the diagram of the lower jaw at the three different timeframes. Sorting through the mutations denoted by asterisk at a given locus to find the particular mutation responsible for lower jaw phenotypic differences is most difficult when species have diverged for a long time (**A**). Even when species have diverged for a shorter time (**B**), the true mutation in the sequence responsible for the phenotypic difference, the large T and large C, is one of three mutations between the two alleles. These other mutations might not affect the size or the mechanics of the lower jaw at all, but their proximity to the true mutation makes isolating the one influential mutation difficult. However, if the species adaptively radiated and their lower jaws rapidly diverged (**C**), the only mutation in their alleles might be the one mutation responsible for the phenotypic differences in the lower jaw. This is why adaptively radiating species make isolating the genetic basis of phenotypic differences more tractable.

When biologists discuss cryptic species they generally refer to recovering extensive genetic differences between groups that are difficult to distinguish phenotypically. For example, many clades of fish in eastern North America contain cryptic monophyletic groups when examined with neutral genetic markers in a phylogenetic context (Egge and Simons 2006; Hollingsworth and Near 2009). These evolutionarily distinct groups show little to no phenotypic differentiation (Fig 2). However, their populations have likely diverged over a substantial amount of time and most regions of their genomes exhibit fixed differences. In these phenotypically cryptic species, even if there were subtle differences one wanted to examine via a genetic cross, reproductive incompatibilities fixed through drift over time would make this difficult. Alternatively, clades that have adaptively radiated could be considered as containing genetically cryptic species. These species show extensive phenotypic differences that allow species to be readily categorized as different evolutionary lineages. However, these species do not exhibit a substantial number of fixed differences in DNA sequences that would allow them to be categorized as genetically monophyletic (Loh et al. 2008). They are likely reproductively isolated in the wild because of color or other prezygotic isolating mechanisms, but have not had time to evolve postzygotic incompatibility (Kornfield and Smith 2000). Therefore, like strains of zebrafish or mice, many genetically cryptic cichlid species can be readily hybridized in the laboratory (Albertson et al. 2003; Lee et al. 2005) to make genetic crosses.

The lack of fixed genetic differences among species of cichlids in places like Lake Malawi and Lake Victoria also makes using phylogenies to study adaptation difficult (Hulsey et al. 2007). However, the lack of fixed differences at neutral alleles could be exploited as null distributions in a population-genetics framework to contrast with fixed differences associated with adaptive phenotypes (Loh et al. 2008). In most groups, the process of genetic drift is ultimately responsible for substantial amounts of genetic divergence and some reproductive isolating mechanisms among species (Uyeda et al. 2009). Selective sweeps, in which stretches of the genome become fixed because of physical linkage to advantageous mutations, can also quickly fix genomic differences (Charlesworth et al. 1995), especially in adaptively diverging lineages. Difference in the rates of fixation between these processes can be used to study associations between genetic changes and divergences in adaptive traits in groups like cichlids.

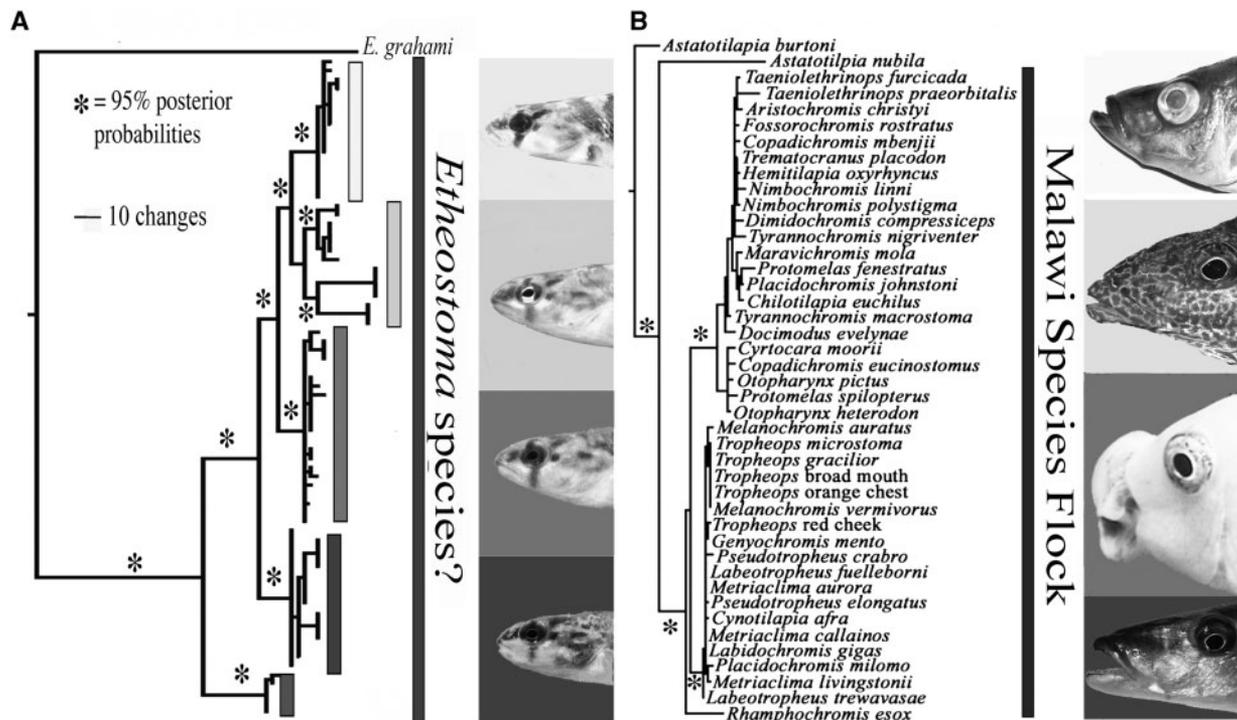


Fig. 2 Two kinds of cryptic species. Generally, when systematic biologists discuss cryptic species they refer to the common phenomenon of finding extensive genetic differences between groups that are difficult to distinguish phenotypically. Many clades of fishes in North America, such as species of darters in the genus *Etheostoma*, exhibit these types of “phenotypically” cryptic species. When putatively neutral genetic markers like the mitochondrial cytochrome *b* gene have been examined among populations (**A**) of a single morphologically diagnosable species, cryptic monophyletic groups are often recovered. These groups often exhibit substantial support denoted by asterisk in the form of genetic differences for their evolutionary distinctiveness. However, these groups show little to no difference in phenotypes like craniofacial morphology, but monophyletic populations inhabiting different river drainages likely have evolved independently for a substantial amount of time. Major river drainages containing monophyletic clades are delineated with the five solidly shaded bars that match the background of the darters’ craniofacial photos. Alternatively, clades that have adaptively radiated often contain “genetically” cryptic species. These species show extensive phenotypic differences that allow them to be readily diagnosed as reproductively isolated lineages. However, these species, like many members of the Lake Malawi species flock (**B**), do not exhibit fixed or substantial differences in DNA sequence. The entire radiation of ~1000 cichlid species in Lake Malawi show substantial craniofacial diversity but exhibit less divergence in mitochondrial DNA sequence than does the one morphologically diagnosable species of *Etheostoma* depicted here.

In recently diverged groups, like human populations or Lake Malawi cichlids, methods known as association mapping have been developed that screen large numbers of genetic loci for associations with quantitative differences in phenotypes (Balding 2006). To clarify how association mapping works, consider an example using a genetic predisposition toward high white blood cell (WBC) count. Although these types of quantitative traits have substantial environmental contributions among individuals, association methods generally ignore that contribution. They focus on the potential relationships between a large number of genetic loci and quantitative phenotypic differences like WBC count. Most loci in human genomes should have no association with WBC count. However, loci that are responsible for this trait, or are physically close on a chromosome to these loci, should

exhibit fixed differences in individuals having a high or low count. Combined with genomic information about gene position and physical linkage that comes from fully sequenced genomes, this association can be used to find candidate loci for quantitative traits among populations (Lynch 2007). As genomic information accumulates, association mapping will allow us to understand how genotypic changes map to adaptive phenotypic changes in recent adaptive radiations like sticklebacks and East African cichlids.

Cichlid adaptation: Linking genomes to phenotypes

One of the keys to understanding how genomic variation could influence adaptive radiations is to document which phenotypes are rapidly diversifying.

In cichlids, most phenotypic studies have centered around three broad types of highly divergent adaptive phenotypes: (1) trophic morphology, (2) color and vision, (3) and reproductive behavior. Divergence in cichlid jaws and teeth is extensive and because of their clear ecological role is generally one of the most-cited examples of cichlid adaptive divergence (Schluter 2000). The co-evolved traits of coloration and the visual system have also long been considered as critical in the diversification of cichlids (Kocher 2004). Finally, the genetics and physiological basis of mating systems, ranging from sex-determination to hormones to neural networks, is increasingly being studied in these fish (Pollen and Hofmann 2008). When compared to other groups of fish, the unparalleled trophic diversity (Fryer and Iles 1972), species richness (Greenwood 1964), and mating behaviors (Kornfield and Smith 2000) of cichlids suggest that some mechanism that links these phenotypes could be critical for explaining their diversification. Understanding the mechanistic basis of how these well-studied traits have changed is also providing insight into how genomes structure the co-evolution of phenotypes during adaptive radiations.

Vertebrate teeth provide an excellent phenotype that integrates across disciplines ranging from ecology to genomics. Tooth shape is used to identify species (Smith and Sansom 1997), infer feeding ecologies (Purnell et al. 2006; MacFadden 2008), and is a model for the interaction of tissues, cells, and genes (Smith 2003; Stock 2007; Fraser et al. 2008). Because of their diversity in the size, shape, and number of teeth, cichlids are an exceptional group for examining which genomic factors promote and constrain the diversification of teeth (Streelman and Albertson 2006). For example, although mammals such as the dolphin *Stenella longirostris* have up to 252 teeth, cichlids can have over 1000 teeth on each of their jaws (Fraser et al. 2009). As many other model vertebrate organisms like mice, chickens, and zebrafish either completely lack or have greatly modified teeth in the oral jaw, there is a need for new model organisms like cichlids in which the developmental genetics of teeth could be studied (Fraser et al. 2008). Low coverage sequencing of several Malawi cichlid genomes, has already provided the sequence data necessary to develop markers for *in situ* hybridization and real-time PCR that can examine tissue-specific gene expression. These gene-expression studies have provided insights into the origin and conserved developmental network responsible for tooth formation in all vertebrates (Fraser et al. 2009). Surprisingly, teeth on both the oral

and pharyngeal jaws of cichlids share a core dental network of gene expression and tooth number is correlated on the oral and pharyngeal jaws (Fraser et al. 2009). These findings demonstrate the utility of cichlids as a model system and provocatively suggest that developmental genetic constraints may heavily influence the diversification of teeth on the jaws of cichlids.

Modularity, or the lack thereof, in phenotypes like jaws and teeth may be critical for dictating patterns of evolution at many levels of biological organization (Liem 1973; Albertson and Kocher 2006; Hulsey et al. 2006). Other seemingly highly integrated elements of cichlid trophic morphology are likely genetically, developmentally, and evolutionarily modular. The genomic modularity of the elements of the lower jaw has been examined using a genetic cross between two morphologically divergent Malawi cichlids (Albertson et al. 2003, 2005). Using this hybrid cross combined with new genetic tools, Albertson et al. (2005) demonstrated that mechanically distinct elements of the lower jaw that includes both the cichlid dentary and the articular share numerous quantitative trait loci (QTL). This suggests that the same regions in the cichlid genome control the size of both elements. These genomically integrated structures stand in contrast to opening and closing in-levers of the lower jaw that share no QTL and can likely evolve independently of one another (Albertson et al. 2005). Interestingly, the jaws and teeth of vertebrates like cichlids also seem to be genetically modular because these elements have different developmental origins and teeth can be readily lost during evolution (Stock et al. 2006). Further examination of this developmental decoupling (Hulsey et al. 2005b) in cichlids could provide insights into the mechanisms controlling the adaptive co-evolution of vertebrates' jaws and teeth.

Foraging behavior, exploitation of different lake microhabitats, and communication among individuals all influence cichlids' visual abilities (Terai et al. 2002; Carleton et al. 2005). In terms of its molecular basis, color perception is one the best-understood traits in cichlid evolution. As in all vertebrates, color perception is mediated through retinal photoreceptor cells containing visual pigments that largely consist of different types of opsins (Yokoyama 2000). Vision over a broader range of wavelengths is accomplished through expression of several opsin genes that are each tuned to different wavelengths of light. The majority of vertebrates have at least one gene from each of the five opsin classes (Yokoyama et al. 1998; Yokoyama et al. 2008).

In cichlids, eight opsin genes have been identified and the opsins that absorb the extreme ends of the light spectrum are the most genetically variable (Terai et al. 2002; Spady et al. 2006). Cichlid species differ in which sets of these genes they express. During ontogeny, the full array of visual pigments in the genome is modulated with the reduction or up-regulation of particular genes establishing unique palettes of visual sensitivities (Parry et al. 2005; Carleton et al. 2008). These suggested regulatory changes could be a key to cichlid visual sensitivities. However, these visual sensitivities can also show large shifts between species through alterations in the amino-acid sequence of visual pigments. For example, adaptive molecular evolution in visual pigments, like rhodopsin, that influence light sensitivity in low- or high-light environments has occurred repeatedly in cichlids inhabiting the substantial depths of the African Great Lakes (Sugawara et al. 2002, 2005). The relative importance of protein coding versus regulatory changes during adaptive evolution is often debated in vertebrate developmental genetics (Streelman et al. 2007), and the visual system of cichlids may offer an exceptional natural system for evaluating the relative importance of these two genomic sources of adaptation.

The mechanisms of adaptation in sensory systems is made even more interesting when examined within a signal-receiver context. Because color allows cichlids to blend into their environment, some patterns of pigmentation clearly have evolved as a result of occupying particular habitats (Fryer and Iles 1972; Seehausen et al. 1999; Maan et al. 2008). However, divergent natural selection on the visual system could clearly interact with how females perceive changes in males' breeding coloration (Maan et al. 2006; Seehausen et al. 2008). Other color patterns have likely evolved specifically in relation to mating. For example, the egg-dummies of males in the haplochromine cichlids of the three Great Lakes mimic eggs and play an important role during courtship of mouth brooding cichlids (Salzburger et al. 2007). Other color phenotypes like the ubiquitous orange-blotch color pattern that occurs in all three lakes appear to be preferentially expressed in females (Streelman et al. 2003). However, it occasionally is expressed in males and disrupts their normal breeding pattern as well as potentially female choice. Our increasing understanding of cichlid genomes should enhance our ability to determine whether the alleles controlling these types of similar color patterns have evolved repeatedly or are simply retained through

evolutionary time. Also, it should elucidate whether clearly co-evolved opsins and pigment genes that influence female choice might be most commonly linked via pleiotropic selection on reproductive behaviors, shared developmental processes, or physical connections to reproductively critical genomic regions like sex-determining loci (Streelman et al. 2003).

Virtually all vertebrates exhibit a reproductive system in which individuals exhibit alternative morphological, physiological, and behavioral differences that are fixed between males and females of a species. However, the mechanisms underlying the determination of sexual phenotypes are diverse and extremely labile (Bull 1983). Unlike birds and mammals (Nanda et al. 2000), few teleosts have karyotypically distinct sex chromosomes (Devlin and Nagahama, 2002). In most species of fish, the sex chromosomes are still in early stages of differentiation (Volff et al. 2007). In medaka and stickleback, the primary sex-determining chromosomal regions have been identified (Matsuda et al. 2002; Kondo et al. 2004; Peichel et al. 2004), but comparatively little is known about the sex-determination system in the zebrafish or the pufferfish. In cichlid fishes, there are at least four distinct sex-determining loci (Lee et al. 2005; Cnaani et al. 2008). The rapid evolution of novel sex-determining loci or the physical re-arrangement of these loci within the genome should readily facilitate reproductive isolation among cichlid species. Additionally, because there is often suppression of recombination on the chromosomes surrounding sex-determining genes (Bergero and Charlesworth 2009), genomic linkage with novel sex-determiners could facilitate the rapid evolution of phenotypes genetically linked to these regions. Furthermore, with the ever-increasing availability of cichlid genomic resources, uncovering which genetic elements vary and which components are conserved during rapid evolution of new sex-determining mechanisms should shed light on sex-determination not only in cichlids but also within all vertebrates.

Nowhere is the complexity of cichlid behavior better illustrated than in the reproductive interactions among individuals. Cichlids exhibit territoriality, sneaker males, single and bi-parental care, bower building, substrate spawning, as well as mouth brooding both in males and in females (Fryer and Iles 1972; Salzburger 2009). As in all vertebrates, cichlid brains and behavior are sculpted by both their genotypes and environment (Pollen and Hofmann 2008). African cichlid fish such as *Astatotilapia burtoni* have become an important model for elucidating the mechanisms underlying

behavioral change and its influence on neural properties and neuroendocrine responses (Fernald 1977; Hofmann et al. 1999; Renn et al. 2004). The development of genomic resources such as BAC and EST libraries (Lang et al. 2006, Salzburger et al. 2008; Renn et al. 2008) have facilitated the construction of microarray panels that provide the means to simultaneously examine the up- and downregulation of hundreds of genes that could influence behavior simultaneously. With these tools, we are learning how ontogenetic changes in behavioral modes, like social status, can alter a wide range of molecular, neuroanatomical, endocrine, and behavioral characteristics (Fernald 2003; Burmeister et al. 2005; Renn et al. 2008) over very short timescales. The great diversity of cichlid reproductive modes, when combined with new genomic tools, will greatly extend our understanding of the genes controlling vertebrate reproduction.

There are many other less-studied phenotypes that have likely adaptively diverged in cichlids and will benefit from a genomic perspective. Although structures such as jaws and teeth have dominated discussions of trophic adaptation, physiological traits such as digestion and growth (Sklan et al. 2004) would be extremely fruitful areas of study in cichlids. This is true both because of the value of *Tilapia* spp. to applied aquaculture and the fact that the diversity in ways of capturing and processing prey that occurs within cichlid radiations undoubtedly results in substantial divergence in these postcranial components of feeding. *Tilapia* spp. are also a model system in studies of respiration and ion-balance (Rengmark et al. 2007; Inokuchi et al. 2009), but these phenotypes would also be interesting to study in cichlid groups that have adaptively radiated. Furthermore, although many fundamental studies examining the mechanics of feeding have utilized cichlids, with notable exceptions (Higham et al. 2007), few studies have examined the substantial functional diversity in aquatic locomotion that likely exists in cichlids. Despite the emphasis on color and vision in cichlid biology, our understanding of the mechanistic basis of other sensory modalities such as olfaction (Miranda et al. 2005) and sound production (Simoes et al. 2008) might be just as critical for understanding diversification of cichlids. As we expand the array of genomic tools available to study cichlid diversification, the ability to answer basic questions concerning the adaptive and conserved elements of the links between genes, development, and phenotypes will also become more tractable.

Utility of cichlid convergence

The genomic era of biology promises not only to provide insight into the mechanistic nature of adaptations, but perhaps more powerfully has ushered in a greater appreciation for the conservation of much of the basic blueprints of life. From the ubiquity of the HOX code in establishing the anterior to posterior axis of all animals' body plans (Duboule 1994) to the conserved nature of Bmp2 and Dlx2 expression in the teeth of both mammals and cichlids (Fraser et al. 2009), genomics has changed our perspective on how similar organisms really are. This similarity makes the developmental genetic basis of phenotypes in organisms as evolutionarily disparate as the zebrafish, stickleback, and cichlids relevant to human health and pathologies.

One major problem with studying evolution is that we cannot rewind history and determine how much of present-day diversity is due to past contingency and/or deterministic forces. Much of the diversity of life could be the result of accidental changes that are contingent upon chance events (Gould 1994), or most divergence could be the consequence of selective forces shaping evolutionary outcomes in a deterministic manner. The evolutionary replication provided by repeated radiations like those found in cichlids provide a window into the relative importance of these evolutionary forces (Seehausen 2007). Because evolution has provided us with experimental replicates, we have the power in cichlids to identify both novel and replicated responses to evolutionary pressures. In cichlids, we can ask what genomic changes are not only sufficient but also necessary in order to evolve repeated novelties such as multi-cuspid teeth, bright blue breeding dress, or monogamous bi-parental systems in raising young.

The replicated convergence in cichlids as well as the diversity of these fish suggests that a common genomic or developmental mechanism could be responsible for driving their extensive divergence. One of the fundamental developmental units that all vertebrates share and into which cichlid diversification could provide insight into is the neural crest. The neural crest is composed of a transient embryonic progenitor population of cells that emerge from the dorsal nerve tube during early development (Landacre 1921). The origin of the neural crest might have been the key innovation that led to the adaptive radiation that has allowed vertebrates to dominate aquatic and terrestrial environments for the past 500 million years (Donoghue et al. 2008). Phenotypes derived from the neural crest include

skeletal and connective tissues, pigment-containing cells, and sensory neurons (Donoghue et al. 2008). These specialized cells and tissues are the developmental progenitors of all of the best studied and perhaps most divergent phenotypes in cichlids, e.g. jaws, color patterns, and reproductive behavior. Therefore, alterations to basic genomic and developmental compartments like the neural crest could frequently mediate the co-evolution and convergence of critical phenotypes during the diversification of cichlids and all other vertebrates (Hulsey et al. 2005b; Brugmann et al. 2006; Kuraku and Meyer 2008).

The replicate nature of cichlid radiations, as well as the broad and rapidly expanding knowledge of cichlid genomics, make these fish an ideal system to begin to mechanistically understand how disciplines as disparate as genomics and ecological change could be linked. Only a few years ago, it would have been difficult to believe that our understanding of the fundamental DNA blueprint represented by the genomes of organisms like cichlids would become so readily available. However, there are a huge number of connections that remain to be made in order to understand how a genome is translated through development into the phenotypic changes that we find so compelling as evidence of adaptive radiation. Perhaps, the most exciting thing that we are discovering with the advent of genome sequencing is that even with a knowledge of the identity of every one of the billions of base pairs in the genome of a cichlid, our understanding of the mechanisms responsible for cichlid diversification has only just begun.

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