

Genomics and vertebrate adaptive radiation: a celebration of the first cichlid genome

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The symposium “Genomics and Vertebrate Adaptive Radiation: A Celebration of the First Cichlid Genome” was held at the annual meeting for the Society of Integrative and Comparative Biology in Boston in January, 2009. While the central topic was the genomics of adaptive radiation in cichlid fishes, the symposium integrated speakers studying genotype-to-phenotype maps in other model organisms. Genomic studies investigating cichlid phenotypes were paralleled with studies using similar techniques to answer questions in other adaptive radiations. The topics were not restricted to any one research area in order to foster interdisciplinary discussions from developmental, physiological, and behavioral research with the common goal to apply and enhance genome-level analyses. Research programs such as the genetics and development of craniofacial diversity, behavior, and color patterns in zebrafish, mouse, and stickleback were described and then augmented with speakers focusing on the genetic and developmental architecture of cichlids’ jaws, behavior, and color. This integration across both established model systems and the new cichlid model sought to capitalize upon a comparative organismal framework that focused on how linking genomic information to studies of phenotype could hasten scientific discovery.

The comparative and integrative approach inherent in most cichlid research extends to the current genome sequencing effort. Multiple cichlids have been chosen for genome sequencing because of their important applied as well as scientific value. The genomes of the cichlid fish *Tilapia nilotica* and as many as three other species representing the faunas of the largest East African Rift lakes will be sequenced. *Astatotilapia burtoni* could represent the 400 species present within Lake Tanganyika and its

surrounding rivers because it is a genomic model for social regulation of behavior (Renn et al. 2008). *Paralabidochromis chilotes* could provide a representative for the ~350 species from Lake Victoria, a radiation being strongly affected by ecological change (Seehausen et al. 2008). *Metriaclima zebra* could also be sequenced because it has been used to build a haplochromine linkage map (Albertson et al. 2005) and is a member of the ~500–1000 species in the very recent radiation from Lake Malawi. These three other cichlid species, representing independent radiations within the East African Rift Lakes, span the incredible craniofacial, color, and behavioral diversity of the nearly 2000 species that inhabit this region (Salzburger 2008). *Tilapia nilotica* will be the first cichlid genome completed, and it is not only a major source of protein for much of the world but also is a focal organism for comparative renal physiology, ion balance, and endocrinology (Rengmark et al. 2007; Inokuchi et al. 2009). The sequencing of these cichlid genomes will provide an unprecedented view simultaneously into the genetics of one of the world’s major protein sources and into the unequalled phenotypic diversity of these fishes. In the symposium, researchers studying cichlids and other model vertebrate organisms focused on exploring the ways in which genomic studies can inform our mechanistic understanding of adaptive phenotypic diversity.

The repeated phenotypic convergence in cichlid fishes makes this diverse vertebrate group exceptional for comparative analyses of genetics and development. Dr Darrin Hulsey described how cichlids are an ideal group to examine the similarities and differences in genomic and developmental changes that have lead to repeated convergence in neural-crest-derived attributes as diverse as jaws, color,

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and social behavior. Because of this evolutionary replication of phenotypes, cichlids provide phylogenetically independent lineages for the examination of complex problems such as how genomic changes map onto developmental alterations that ultimately lead, via nonlinear pathways, to adaptive phenotypic change (Hulsey et al. 2005). The rate of phenotypic divergence in cichlids was also highlighted and the usefulness of such rapidly accumulating phenotypic diversity for genetic studies using hybrid crosses and techniques, such as admixture mapping, were discussed. The accessibility of genome sequencing, how genomic information can be applied to understanding organismal phenotypes, and how this information can refocus old hypotheses and pose novel ones was explored using cichlids as a model system.

The genome sequencing of nontraditional model organisms that are members of adaptive radiations such as *Anolis* lizards of the Caribbean, the threespine stickleback, and the African cichlids is in progress. Dr Kerstin Lindblad-Toh from the Broad Institute discussed technological advances in our ability to rapidly sequence and assemble genomes that are enabling the genomic blueprint of an incredible diversity of species to be obtained. The realization that traditional model organisms have limitations for understanding the genotype-to-phenotype map make genetic information from a diverse array of vertebrates attractive for use in studies both of human disease and evolutionary divergence (Lindblad-Toh et al. 2005). The availability of complete genome sequences for these species will reveal valuable information on the size, structure, and content of vertebrate genomes. Furthermore, it will allow the development of genomic tools that can facilitate our understanding of the genomic basis of adaptive phenotypic change as well as of the interaction between adaptation and genomic processes such as recombination rates, pleiotropy, and chromosomal structure. Specific examples were discussed of how output from genome-sequencing projects such as single nucleotide polymorphisms (SNPs) leverage science in nontraditional model species and simultaneously facilitate the study of comparative genomics and evolution in adaptive radiations.

The evolutionary history of East African cichlid fishes is characterized by repeated bursts of skeletal change associated with modifications for trophic specialization. Dr Craig Albertson discussed how this rapid divergence in the jaws, teeth, and cranium of cichlids has led to the evolution of dramatically different craniofacial morphologies. Because of their

recent divergence, many cichlids that differ substantially in craniofacial phenotypes can be hybridized in the laboratory. With the use of markers developed in the past as well as novel markers obtained from drafts of these cichlid genomes, the association of phenotypes to particular genomic regions can be examined in genetic crosses formed between trophically divergent cichlid species (Albertson et al. 2005). Using a hybrid cross between two species of cichlids from Lake Malawi, *Metriaclima zebra* and *Labeotropheus fuelleborni*, the genetic basis of several craniofacial features like jaw lever systems and bone fenestration have been examined. Interestingly, the genomic regions associated with QTL (quantitative trait loci) for several of these skeletal elements exhibit a significant degree of genetic and developmental integration. The rapid and replicate nature of cichlids' trophic evolution may be due, in part, to genomic patterns of integration among trophic characters. The addition of cichlids to the growing number of vertebrate species whose genomes have been sequenced will provide an unprecedented opportunity to test novel hypotheses about craniofacial evolution.

Dentition in fishes exhibits enormous diversity in shape, number, and location of individual teeth, and because of this diversity, teeth provide an exceptional vertebrate system that can be used to examine the developmental basis of novelty (Stock 2007). Dr David Stock discussed how his laboratory is carrying out comparative analyses of tooth development focused on the zebrafish, *Danio rerio*, a species around which unparalleled genetic, molecular, and embryological tools for the analysis of development have been created. One of the most distinctive features of dentition in the zebrafish is the restriction of teeth to a single pair of elements, the fifth ceratobranchials, as a result of evolutionary reduction of tooth-bearing locations. To understand the mechanisms of this reduction, he discussed developmental comparisons between zebrafish and the characiform Mexican blind cave tetra, *Astyanax mexicanus*. These comparisons of gene expression, coupled with mutant analyses in the zebrafish revealed several candidate genes involved in reduction of the dentition. Transgenic and pharmacological manipulation of the expression of genes in the zebrafish and *Astyanax mexicanus* produced altered numbers of teeth and of tooth cusps. These results provide insight into the mechanisms leading to tooth reduction in cypriniforms and the origins and ontogenetic distribution of multicuspid teeth in fishes.

One of the textbook examples of cichlid phenotypic diversity is their continuously replacing oral and

pharyngeal jaw dentitions that exhibit dramatic phenotypic variation. Dr Todd Streelman highlighted the utility of genomic studies in new model organisms with a recent study on the development of the oral and pharyngeal jaw dentitions of Lake Malawi cichlids (Fraser et al. 2009). He argued that much of our understanding of the developmental genetics of vertebrate phenotypes stems from genetic screens of highly inbred organisms that, because of mutations, may have lost traits or exhibit traits that are highly aberrant. He discussed how cichlids and other rapidly radiating groups alternatively offer phenotypic variation that is the result of developmental and genetic changes resulting from functional adaptive processes. He further demonstrated how much of the genetic variation that exists within a particular species flock may also segregate in other closely related cichlid flocks. This suggests markers and methods developed for fish in one of the African Rift lakes may frequently be useful to examine the developmental and genetic basis of phenotypes in other cichlids. These studies of cichlid dentitions highlighted the amazing modularity of jaws and teeth as they have coevolved during the history of vertebrates. This theme vividly displayed how genome sequences will usher in a better understanding of how novelty arises as well as which developmental components of anciently derived vertebrate structures, such as teeth, are conserved.

The recent genomic sequencing of the stickleback, *Gasterosteus aculeatus*, has provided an innovative view into the evolution of one of the model systems of adaptive radiation. Integrating genomic and ecological approaches into the study of stickleback evolution, as discussed by Dr Janette Boughman, promises great advances in our understanding of how adaptive divergence occurs on a microevolutionary level. With the explosion of genetic resources for threespine sticklebacks, including a complete genome sequence, we can now untangle the genetic basis of traits conferring premating isolation as well as adaptation to distinct environments (Boughman et al. 2005). This talk focused on the genetics of males' color, that previous work demonstrated was adapted to distinct light conditions in the mating habitat of two stickleback species: (1) a limnetic species occupying the water column and (2) a benthic species largely exploiting the bottom of lakes for food. Similar to cichlids, the process of sensory drive (Seehausen et al. 2008) has likely contributed to divergence in color and in the perception of color between species and could be driving the evolution of premating isolation in sticklebacks. Using genomic markers and natural populations of a hybridizing

species pair, an approach based on admixture mapping has been employed to examine the genetic basis and physical linkage in the genome of traits involved in premating isolation. This ongoing work combines experimental and genetic studies on the nature of selection acting on the suite of traits that confer premating isolation. The results will provide an excellent comparison to those obtained in cichlid systems with corresponding divergence of traits.

The visual system is a critical mechanism that during vertebrate diversification has driven both trophic diversification and the interaction of the sexes through mate choice and display. Dr Karen Carleton discussed diversity in the cichlid visual system that exhibits some of the largest known differences in visual sensitivities amongst closely related species. Cichlid fishes are well-known for their diverse color patterns and breeding systems and these phenotypes co-evolve extensively with changes in the visual system of cichlids (Carleton et al. 2008). Diversity of the cichlid visual system arises in part because these fish have seven unique cone opsin genes that are sensitive from the ultraviolet to the red ends of the spectrum. Because species differ in which sets of these genes they express, visual sensitivities can show large shifts between species that can be finely tuned through alterations in opsin amino-acid sequence. Genome sequences will further extend the progress made in determining the molecular mechanisms controlling cichlid visual sensitivities and elucidate vision's role in driving cichlids' reproductive diversification.

Sex determination is a fundamental developmental decision in most organisms wherein individuals are directed down specific pathways of male or female reproductive function. Dr Thomas Kocher discussed our understanding of the genetic basis of sex determination in cichlids. He discussed how the traditional focus on mammalian genetic systems often misleadingly gives the impression that sex-determination is largely immutable during evolution. However, outside of mammals and birds that exhibit obvious chromosomal sex-determination, vertebrates exhibit an incredible array of sex-determining mechanisms. In cichlid fishes, including cultured tilapias and the haplochromine cichlids of East Africa there are at least four distinct sex-determining loci (Cnaani et al. 2008). With the ever-increasing availability of cichlid genomic resources, uncovering which genetic elements vary and which components are conserved during the evolution of this group should shed light on sex-determination not only in cichlids but also within all vertebrates.

The house mouse, *Mus musculus*, is the best genomically characterized and most extensively used vertebrate in examinations of the genetic basis of phenotypes. Dr Elissa Chessler discussed how the mouse is rapidly becoming the centerpiece of studies examining vertebrate systems genetics, a biological approach in which allelic variation is studied as natural perturbations of a biological network. Its evolutionary proximity to humans, relatively rapid generation time, and the plethora of genetic resources available to manipulate its genome all insure that the mouse will continue to occupy a primary focus in studies of the link between genotype and phenotype. Recently, advances in genetic analysis, made possible through the mouse genome, have uncovered both the phenomenal potential of systems genetics as well as the current limits to genetic analysis that employ previously bred populations of mice. As a response to these limitations, the Complex Trait Consortium has devised a novel mouse population that exhibits higher allelic diversity, power, precision, accuracy, and independence of recombination than did previous experimental populations of mice (Chessler et al. 2008). Fundamental to many applications in systems genetics, phenotypes in the Collaborative Cross are retrievable and consist of a panel of isogenic lines. This has generated a highly polymorphic population with dense recombination and haplotype breakpoints providing enhanced power to isolate regions of the genome associated with particular phenotypes. The results gained from this genetic cross will also provide insight into how systems genetics can improve the identification of genes coding for traits such as color and behavior in new model genetic systems like cichlids.

Complex brains and behaviors have arisen repeatedly in vertebrate evolution. Dr Hans Hofmann discussed how adaptive pressures drive such changes in brains. He explained the application of genome-level analysis to investigate the molecular basis of physiological mechanisms that underlie complex social phenotypes in nonmodel organisms (Pollen and Hofmann 2008). The diversity of East African cichlid fishes provides an especially valuable opportunity to analyze the social and ecological correlates of neural phenotypes that resulted from rapid, recent, and repeated radiations. Dr Hofmann described how ecological pressures have sculpted neuroanatomical traits that can be examined through genomic studies. He demonstrated how environmental and social factors differentially affect the brain and likened the regulation of pair-bond formation in cichlids to that known in rodents. A similar set

of genes as well as novel genes have been recruited during repeated independent evolutionary transitions of mating phenotypes in cichlids. Studies in cichlids are providing important insights into the molecular and physiological underpinnings of social behavior and its evolution.

Even without full genome sequence, research programs represented by those in this symposium have made great progress through the use of cDNA microarrays and genetic analysis. Dr Suzy Renn presented results on expression results for a novel foray into maternal aggression and care that will parallel the detailed molecular and physiological work that has been carried out on male *A. burtoni*. While 30 years of research have contributed to our understanding of the mechanisms of the socially regulated switch between dominant and subordinate phenotypes in males, the phenotypes of females have been largely ignored. Using comparisons between the sexes of gene expression profiles of aggressive phenotypes, Dr Renn has identified modularity in gene expression related to aggression (Renn et al. 2008). She further intends to use this one cDNA array to explore social regulation of gene expression in other cichlid species for which there is a wealth of behavioral and ecological research. These studies lay the groundwork for a systems-level analysis to address the modularity of gene expression and the evolution of behavior.

This symposium highlighted that even with the sequencing of several cichlid genomes discoveries of the mechanisms responsible for the diversification of cichlids and all other vertebrates have only just begun.

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