Cichlids in the Genomics Era: What can we learn about the genetic basis of their rapid speciation?

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The Bauer Center for Genomics Research at Harvard University brings together a diverse spectrum of scientists, aiming to apply genomics tools to understand complex problems through novel approaches. Our research group, headed by Dr. Hans A. Hofmann, focuses on the various fascinating aspects of cichlid fish biology. Our team includes postdoctoral researchers with backgrounds in neurobiology, evolutionary biology and endocrinology, as well as a molecular biology PhD candidate and a number of undergraduate students, all with a unifying interest in cichlids, specifically the African Cichlids of Lake Tanganyika. The wide diversity of cichlids makes them captivating but also harder to study in a comparative manner due to the sheer number of species that have evolved. Therefore, we would like to enlist the support of the community of cichlid aquarists to reach this new research goal. We have designed a large comparative genomics experiment that involves obtaining DNA from many cichlid species. In much the way that volunteer efforts have helped Ron Coleman and his Cichlid Egg Project (**cichlidresearch.com/eggproj.html**), we need your help.

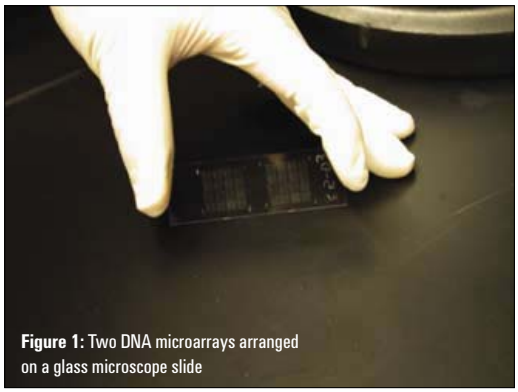

Figure 1: Two DNA microarrays arranged on a glass microscope slide

The term "speciation" refers to the process of how a new species comes to exist. The understanding of this process is one of the central questions in evolutionary biology. The project that we have planned may help to answer this question. Why is one species blue and another brown? Why does one live in rocks and another thrive on sand? Why does one brood its young in the mouth and others guard them on the substrate? What about the bewildering diversity in body shapes, fins, mouthparts, and size found in these wonderful fishes? Many of these differences in morphology and behavior are caused by differences in the genes of these species. For example, while fish have the genes that are necessary to build fins, these genes may have slight differences that cause the fish to sport different appendages. Indeed, just as a wall can be made with many different color or size of bricks and still give a final product that will be very much the same, the different cichlids species probably have many different sizes and colors of bricks to build the spectacular features that we see in each species. So, if they have most of the same genes, can we find the ones that contain changes ("mutations"), which make two species of fish, look and behave so differently? Which genes and just how many of them vary between strikingly different closely related species? This is the question that our research group at Harvard's Bauer Center for Genomics Research wants to answer. And this is where your input becomes crucial.

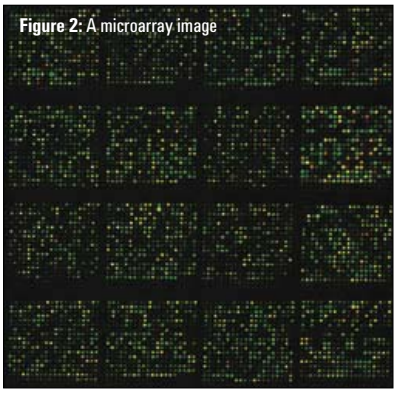

Figure 2: A microarray image

You ask, "how can a hobbyist with a few (or hundreds of) fish in his/her home help with a "genomics" level project?" The answer is simple. All of your fish have DNA in every cell of their body from their nose to their tail. The DNA is the 'code of life' as it contains instructions for what, where and when building blocks will be used to create the fish. A "genome" is made up of many genes. Each gene contains the information to construct a protein. Genes are made of DNA. While the role of DNA in constructing life is very complicated, it really has a very simple basic structure. The instructions held in the gene are coded in an alphabet made of small molecules called "nucleotides" that biologists represent as A, C, G and T. These nucleotides are strung together to form the DNA. We can therefore study the "sequence" of a gene by reading this alphabet. This is why we are so interested in studying the DNA of many different species. Mutations are changes in order of the letters (A, G, C and T) of this alphabet. In some case these mutations affect the structure of the protein, which that gene encodes. It is these changes in sequence that we want to correlate to the differences we can see in the different fish species such as color, shape and behavior.

In our study, we are taking advantage of a new method called a DNA microarray. A microarray is a tool to examine thousands of genes at a time. An array contains thousands of spots. Each spot holds the DNA strands that represent a different gene. Our micorarray contains ca. 3500 different genes. The microarray is about the size of a postage stamp (figure 1) so two can be "printed" onto a glass microscope slide using a precise robot. The microarray can be used in an experiment to identify differences in the DNA of two species.

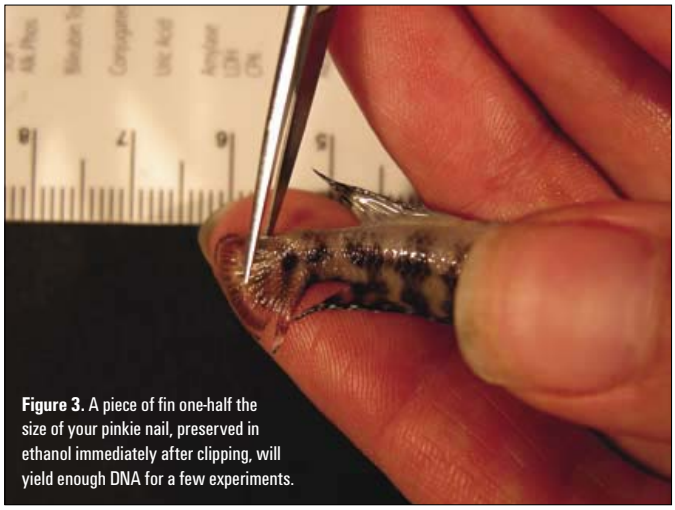

Figure 3. A piece of fin one-half the size of your pinkie nail, preserved in ethanol immediately after clipping, will yield enough DNA for a few experiments.

When the DNA from one fish is incubated on this microarray, it will stick (called "hybridize") to a spot if the sequence of A, C, G and T in the fish matches the DNA in the spot on the array. We can see the hybridization of the fish's DNA on the microarray as a fluorescent glow because we first label the DNA sample from the fish. In an experiment to compare the DNA from one fish species to that of another species the two samples are labeled with different labels that glow with different colors, usually visualized as red and green. We can see if the two species contain very similar DNA sequences because they will both hybridize to the spot equally well. In equal hybridization the red labeled DNA from one fish plus the green labeled DNA from the other species will appear yellow in the resulting microarray image (figure 2). However, if one species has many different mutations in the DNA sequence, so that it does not hybridize well, the spot on the microarray will appear to be biased toward the other color. With this method we can determine if there are differences in the DNA sequences between the two different species, without actually having to read all the sequences individually. Reading the sequence directly is called "DNA sequencing" and it requires a lot of time, money and effort. We believe that a microarray-based approach is an efficient way to compare many cichlid species and many genes in a more efficient manner. This type of experiment is called "high throughput" science.

We will study many species. By comparing the results for many species we hope to identify certain genes that are always different between species with different fin length (long versus short fins) or different parental care (mouth brooding versus substrate guarding) or any other characteristic difference (morph, behavior or habitat) that is interesting. The genes that are identified as always being different between any two species, that are different with regard to the interesting characteristic, may be the genes that are crucial for the difference in this trait.

Our study therefore requires DNA from many different species. We do not need the fish in order to isolate the genomic DNA that we need. We only require a small clip from a fin. A piece of fin one-half of the size of your pinkie nail (figure 3), preserved in ethanol immediately after clipping, will yield enough DNA for a few experiments. In this way biologists can study DNA of precious species that we don't want to harm. We are looking for "fin clips" from many different species. We thought that you, as dedicated aquarists, might have many of the fish that we are looking for. The most important thing that you can supply along with the fin clip is accurate information about the stock, not just genus and species, but from what region of the lake does it originate, and how many generations has it been in captivity. We will supply you with vials and storage solution for the fin clips and would gladly pay shipping costs. As you know, a small clip from the fin will not hurt the fish and will grow back quickly. In the lab we can then isolate the DNA (figure 4) and conduct our experiments. If you are interested in helping our research group, please contact us for more detailed instructions (**harvardcichlid@yahoo.com**). We have quite a list of fish that we want (too many to publish here) that you can find on our lab website. In addition to cichlids from Lake Tanganyika, we are also interested in species from other lakes so please do contact us if you would like to help (**http://www.cgr.harvard.edu/hans/**). We hope to have some exciting results to tell you about the next time we write a paper.

Figure 4: Our lab