

7-18-2003

# Molecular Systematics of a Rapidly Evolving Species Flock: The Mbuna (Cichlidae) of Lake Malawi

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## Recommended Citation

Kornfield, Irving L., "Molecular Systematics of a Rapidly Evolving Species Flock: The Mbuna (Cichlidae) of Lake Malawi" (2003).  
*University of Maine Office of Research and Sponsored Programs: Grant Reports*. 88.  
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**Final Report for Period:** 09/1997 - 03/2001**Submitted on:** 07/18/2003**Principal Investigator:** Kornfield, Irving L.**Award ID:** 9707532**Organization:** University of Maine**Title:**  
Molecular Systematics of a Rapidly Evolving Species Flock: The Mbuna (Cichlidae) of Lake Malawi**Project Participants****Senior Personnel****Name:** Kornfield, Irving**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Graduate Student****Name:** Smith, Peter**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Kuhn, kristen**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Optimized PCR reactions for diverse loci of zebra population; acquired new microsat data.

**Name:** Lage, Christopher**Worked for more than 160 Hours:** No**Contribution to Project:**

Analyzed data sets for new allelic information

**Name:** Haye, Pilar**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Data acquisition for population phenotypes.

**Undergraduate Student****Name:** Reddy, Nirupa**Worked for more than 160 Hours:** No**Contribution to Project:****Technician, Programmer****Other Participant****Research Experience for Undergraduates****Organizational Partners**

Dept. of Fisheries

During Malawi fieldwork for both years, exchanged and identified materials.

### **Other Collaborators or Contacts**

On both visits to Malawi the PI met with a number of officials. Meetings were held with the head and faculty of the Dept. of Biology of the University of Malawi, the chief and deputy chief of the Malawi Department of Fisheries, and with senior members of a major international cichlid project supported by the Southern African Development Corporation (SADC) sited at Salima, Malawi. Ichthyologists at SADC assisted in collection and identification of specimens, and participated in research discussions about cichlid evolution.

### **Activities and Findings**

#### **Research and Education Activities:**

During the period of this award, (1) fieldwork was conducted in Lake Malawi on color variants in the *Metriaclima* (=Pseudotropheus) zebra complex, (2) molecular characterizations of populations of these taxa were obtained using microsatellite loci, and (3) these data were analyzed to test hypotheses concerning the origin of color variants and the power of microsatellites to establish phylogenies.

The PI visited Malawi Sept. 13 - Oct. 19, 1997 and Nov. 7 - 30, 1998, accompanied on the latter trip by Peter Smith, a graduate research assistant. Meetings were held with the faculty of the Dept. of Biology at the University of Malawi, the head of Malawi Department of Fisheries, and with members of a major international cichlid project under the aegis of the Southern African Development Commission (SADC). Fieldwork was conducted in the southern portion of the lake, an eastern portion in Mozambique waters, and a northern portion extending from Nkhata Bay to Culumba. During fieldwork, population samples of 10 taxa (species and allopatric color variants) were collected as well as closely related mbuna. In situ observations were made and recorded for color variation and behavioral interactions. On the second trip, we returned to the US via Mombassa, Kenya where we collected samples of cumaceans for an PEET project.

Extensive molecular characterization of the collected materials was conducted using microsatellites. Genotypic information from a suite of eight loci was obtained for most populations. The benchwork involving DNA extraction, PCR, and microsatellite genotyping for over 1000 specimens was a major enterprise. Details of methodologies appear in our publications.

Information was extracted for population samples from multilocus geneotypes. Analysis of genic and genotypic information was conducted using a wide range of software and approaches. To establish baseline (null) expectations for some hypotheses, extensive simulations of population processes were conducted, i.e., intertaxon gene flow. Details of data analysis is provided in our publications.

Our findings on in situ body coloration, the genetic affinities of allopatric populations of discrete color variants, and the phylogenetic relationships among taxa were established and communicated in the peer literature.

All of the activities above were conducted by students (and by the PI). All acquired backgrounds designed to permit them to formulate and execute rigorous future research in evolutionary biology

**Findings:**

(1) Our catalogues of coloration and barring patterns demonstrated that many of these characters, presumably diagnostic of described species, were widespread among congeners. This information, communicated in our review of cichlid evolutionary genetics, provides caution to taxonomists describing new materials from all of the African Great Lakes.

(2) Our analysis of congruence between color phenotypes and microsatellite genotypes demonstrated, for the first time, convergence in coloration among unrelated genetic lineages. This implies that divergence in coloration may accompany speciation, and that allopatric populations with similar coloration cannot be assumed to be conspecific.

(3) Our analyses of multilocus population profiles identified gene flow between congeners in both the northern and southern populations. These findings suggest that hybridization may be widespread in African cichlid faunas. However, the role of hybridization in speciation is elusive. These findings prompted development of assays to identify cryptic hybrids using measure of linkage disequilibrium for loci selected from extant cichlid maps.

(4) Our phylogenetic analyses using microsatellite data provided a tree with reasonable topology, i.e., consistent with expectations from earlier genetic studies. Bootstrap support for the tree was moderate (50-60%). We conclude that microsatellites can be used for phylogenetic reconstruction in this and other faunas if the number of markers is expanded, and if the markers have information content.

**Training and Development:**

Training has been directly provided to three graduate student and one undergraduate. One of these grad students conducted fieldwork in Malawi and was trained in field logistics and SCUBA work. An additional graduate student participated in the project. Training has been in the following areas: cichlid evolutionary biology, fundamentals of taxonomy, and molecular systematics. Training (more generically, educating) in these and related areas is an on-going process; as new techniques and analytical approaches are implemented, they are shared with all workers in my lab.

**Outreach Activities:**

Because I have extensive contact with undergraduates in my teaching, I have been able to integrate a variety of aspects of this NSF supported research project into my lectures. For example, in BIO 465 (Evolution, annual enrollment ~ 60 students), I use Malawi cichlid fishes as model organisms in discussions of taxonomy (illustrating species concepts as well as formal description), population genetics, and higher level processes. Similarly, this material is used in graduate classes, BIO 540 and SMS 597. Since receiving a major teaching award at the beginning of the award period, I have had the opportunity to meet with a variety of faculty and interest groups. These occasions presented me a forum to explain my research and its broader implications to diverse audiences. Such fora included the University of Maine Alumni Association in addition to regular science seminars on campus.

**Journal Publications**

Kornfield, I. and P. Smith, "African cichlid fishes: model systems for evolutionary biology.", Annual Reviews of Ecology and Systematics, p. 163, vol. 31, (2000). Published

Smith, P. and I. Kornfield, "Phylogeography of Lake Malawi cichlids of the genus *Pseudotropheus*: significance of allopatric color variation", *Proceedings of the Royal Society of London*, p. 2495, vol. 269, (2002). Published

Smith, P.F., A. Konings and I. Kornfield, "Hybrid origin of a cichlid population in Lake Malawi: implications for genetic variation and species diversity", *Molecular Ecology*, p. , vol. , (2003). Accepted

Smith, P. and I. Kornfield, "Extreme color convergence within the *Maylandia* (*Pseudotropheus*) zebra species complex of Lake Malawi cichlids revealed by a microsatellite phylogeny", *Proc. Zool. Soc. Wash.*, p. , vol. , ( ). Submitted

Hooge, M.D., P.A. Haye, S. Tyler, M.K. Litvaitis, and I. Kornfield., "Molecular systematics of the acoelas (Acoelomorpha, Platyhelminthes): polyphyly at the family level", *Mol. Bio. Evol.*, p. 333, vol. 24, (2002). Published

Kornfield, I, "Assessment of hybridization via linkage disequilibrium: benefits of a map", *Molecular Ecology*, p. , vol. , ( ). Submitted

### **Books or Other One-time Publications**

#### **Web/Internet Site**

**URL(s):**

[nature.umesci.maine.edu/peter/petercichlid.html](http://nature.umesci.maine.edu/peter/petercichlid.html)

**Description:**

Provides basic information about the Malawi cichlid system.

#### **Other Specific Products**

**Product Type:** Physical collection (samples, etc.)

**Product Description:**

Tissue samples and whole specimens from population samples of *M. zebra* and other taxa were collected and returned to the US. Voucher specimens for some populations were deposited with the SADC project.

**Sharing Information:**

Tissue samples have been provided to workers at the University of New Hampshire for phylogenetic reconstruction of Malawi cichlids using AFLP. This work is a collaborative effort among UNH, Ole Seehausen's group at the University of Hull, and the PI at the University of Maine.

**Product Type:** DNA sequence

**Product Description:**

Sequences for new microsatellite loci were distributed to Tom Kochers lab for mapping *Tilapia*. Partial mtDNA sequences were obtained for the project Molecular systematics of the acoelas (Acoelomorpha, Platyhelminthes): polyphyly at the family level.

**Sharing Information:**

Sequences were deposited in GenBank (AY078365-).

#### **Contributions**

**Contributions within Discipline:**

Contributions to evolutionary biology have included presentation of findings at the Tropical Fish Biology Symposium, 13-16 July 1998, University of Southampton, UK; see *J. Fish Biol.* Dec. 1998, vol. 53, Suppl. A), Virginia Polytechnic and State University (VPI) Mar. 15, 2001, Marine Biological

Laboratory, Woods Hole, April 18, 2001, and Dartmouth College Apr. 15, 2002. In addition, informal discussions with participants in African cichlid research from other institutions occurred regularly. Based on the work conducted under this award, the PI will speak as an invitee at a special meeting on cichlid research to be held in Toyko, Sept. 15-26, 2003.

**Contributions to Other Disciplines:**

**Contributions to Human Resource Development:**

Activities in Malawi supported by this award have developed linkages with educational missions via presentations to the faculty at the University of Malawi and discussions with interested individuals in other areas. In the US, human development has principally involved integration of graduate and undergraduate students into the research program.

**Contributions to Resources for Research and Education:**

**Contributions Beyond Science and Engineering:**

Prompted by studies of translocations with Lake Malawi, I have developed material relating to ecological disruption as a terrorist strategy. The introduction of exotic macrofauna can potentially disrupt extant aquatic ecosystems, causing local (or global extinctions) and altering environments to new equilibria. If targeted, some commercially important organisms could be effected.

**Categories for which nothing is reported:**

Any Book

Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education