Doctoral Dissertations

Student Scholarship

Fall 2006

Patterns and process during the diversification of the cichlid fishes in Lake Malawi, Africa

Michael R. Kidd
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/dissertation

PATTERNS AND PROCESS DURING THE DIVERSIFICATION
OF THE CICHLID FISHES IN LAKE MALAWI, AFRICA

BY

MICHAEL R. KIDD
BA, Williams College, 1991

DISSERTATION

Submitted to the University of New Hampshire
In Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Zoology

September, 2006

UMI Number: 3231355

INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

UMI®
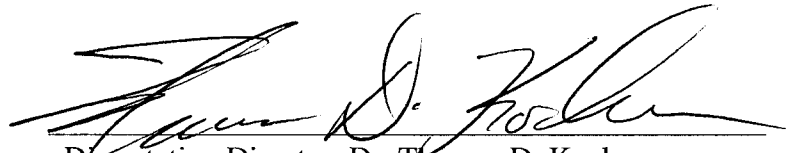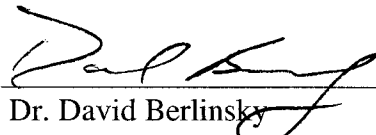
This dissertation has been examined and approved.

Dissertation Director, Dr. Thomas D. Kocher
Professor of Zoology and Genetics

Dr. David Berlinsky
Associate Professor of Zoology

Dr. Karen L. Carleton
Research Associate Professor of Zoology

Dr. Irving Kornfield
Professor of Zoology, University of Maine

Dr. Michelle P. Scott
Professor of Zoology

07/18/06
Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

PATTERNS AND PROCESS DURING THE DIVERSIFICATION
OF THE CICHLID FISHES IN LAKE MALAWI, AFRICA

BY

MICHAEL R. KIDD

UNIVERSITY OF NEW HAMPSHIRE, SEPTEMBER, 2006

The 500-1000 cichlid species endemic to Lake Malawi constitute one of the most

rapid and extensive radiations of vertebrates ever discovered. The objective of this

dissertation was to test the assumptions and predictions of several recently published

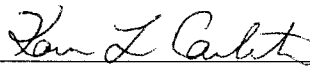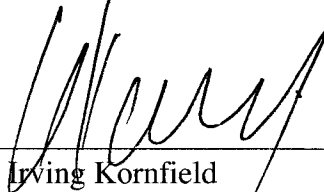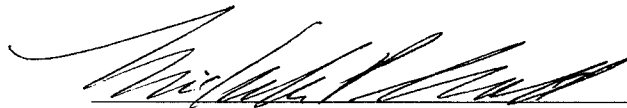models of cichlid speciation. First, a novel assay of female choice was used to evaluate

the role of visual cues during conspecific recognition. The results of this experiment

demonstrate that females are able to identify conspecific mates using only visual cues.

Second, the assumptions of a recent model of speciation via intrasexual selection were

tested by comparing aggressive behavior during territorial contests among five closely

related cichlid species from Lake Malawi. I found that interactions between

conspicuously heteromorphic males tend to be less aggressive than between those of

homomorphic males. Third, I used transect data to evaluate the intrinsic and extrinsic

factors that have affected the dispersal of 17 closely related cichlid species introduced to

Thumbi West Island in Lake Malawi. A quarter of a century after their initial

introduction, I found that the translocated cichlid community around Thumbi West Island

is still in a state of flux, as many species continue to spread around the island. Next, I evaluated the patterns of divergence predicted by three competing speciation models that differ in the temporal action of natural and sexual selection by using AFLP to reconstruct the evolutionary relationships among 20 species of the ubiquitous rock dwelling subgenus *Pseudotropheus tropheops*. I found that cladogenesis is associated with numerous unambiguous changes in male color states. A geometric morphometric analysis of the lower jaw of these taxa revealed that the repeated differentiation of jaw morphology is associated with species habitat preference. Finally, I used AFLP data to reconstruct the evolution of species within three genera of sand dwelling cichlids that construct elaborate male display platforms. I found that sister taxa with distinct bower morphologies, and that exist in discrete leks separated by only 1-2m of depth, are divergent in both sexually selected and ecological traits. These phylogenies suggest that the forces of sexual and ecological selection have been intertwined during the speciation of these groups.

INTRODUCTION

The cichlids of East Africa's Great Lakes are an unparalleled example of explosive speciation. Lakes Tanganyika, Malawi and Victoria each contain independent radiations of 250 to 1,000 endemic cichlid species, which display a staggering variety of behaviors, color patterns, and trophic adaptations and make them an invaluable model system for evolutionary biologists (Kornfield & Smith 2000, Genner & Turner 2005). Within Lake Malawi over 700 species have evolved from a single common ancestor within the past 2 million years (Meyer *et al.* 1990). Recent molecular evidence suggests that the diversification of this remarkable lineage has occurred even faster than previously estimated and that much of its current cichlid fauna formed within the last 10,000 years (Won *et al.* 2005). Molecular data suggests that Lake Malawi's cichlid flock contains two primary lineages consisting of rock dwelling (mbuna) and sand dwelling (utaka and chisawasawa) species. The monophyletic nature of both lineages has been confirmed by mitochondrial DNA (Kocher *et al.* 1995, Salzburger *et al* 2002) and nuclear sequence (Mayer *et al.* 1998) and SINE (Takahashi *et al.* 2001) insertion data. The endemic cichlid flocks of the African Great Lakes represent a challenge for biologists seeking to identify the forces that generate and maintain this circumscribed vertebrate radiation 20-400 times more diverse than Darwin's finches.

While many models have been designed to enumerate and elucidate the evolutionary forces underlying cichlid speciation (Mayr 1984, Wu 1985, Turner & Burrows 1995, Higashi *et al.* 1999, Kondrashov and Kondrashov 1999, Takimoto *et al.*

1

2000, Danley & Kocher 2001, Lande *et al.* 2001, Kocher 2004, Seehausen & Schluter 2004), testing these models is often difficult because of the long time scales over which new species typically form. Additionally, characters important for current reproductive and ecological discrimination may not be related to the factors that initially promoted population divergence (Coyne & Orr 2004), especially in a climatically dynamic region where lake level fluctuations have the potential to rapidly change environmental conditions and species distributions (Owen *et al.* 1990, Johnson *et al.* 2000, Sturmbauer et al. 2001). Recent discussions of cichlid speciation have focused on the relative role of natural and sexual selection during the explosive radiation of these remarkable species flocks (Danley & Kocher 2001, Salzburger & Meyer 2004, Arnegard & Kondrashov 2004, van Doorn *et al.* 2004, Genner & Turner 2005). Unraveling the forces acting during cichlid speciation requires stringent behavioral and morphological assays coupled with robust phylogenetic reconstructions of recently evolved groups of species.

Mayr (1984) lamented that morphology based cladistic analysis of East African cichlid species was inadequate to generate robust hypotheses of evolutionary history and thus precluded our understanding of the mechanisms involved during speciation. Phylogenies based on morphology are limited by the lack of appropriate synapomorphies (Stiassny 1991) and the rampant convergence of morphological characters between (Kocher *et al.* 1993, Rüber *et al.* 1999) and within lakes (Reinthal & Meyer 1997, Kidd *et al.* 2006b). The advent of molecular techniques revitalized the study of cichlid speciation by demonstrating the reciprocal monophyly and rapid evolution of the Lake Victoria and Lake Malawi species flocks (Meyer *et al.* 1990, Kocher *et al.* 1993, Moran *et al.* 1994). While mitochondrial sequence data has been invaluable in reconstructing the

2

relationships between the older and more divergent cichlid species within Lake

Tanganyika (Nishida 1991, Sturmbauer & Meyer 1993, Sturmbauer *et al*. 1994,

Verheyen *et al*. 1996, Baric *et al*. 2003), its usefulness in discerning the evolutionary

history of the more recently diverged Lake Malawi and Lake Victoria species flocks has

been confounded by the retention of ancestral polymorphisms (Moran & Kornfield 1993,

Parker & Kornfield 1997),

A multilocus approach is necessary in order to overcome the confounding

influence of ancestral polymorphisms (Won *et al*. 2005, Kocher 2003) and to evaluate the

behavioral, morphological and ecological characters promoting population divergence.

Microsatellites have proven useful for identifying the relationships between closely

related species and populations (Arnegard *et al*. 1999, Markert *et al*. 1999, Danley *et al*.

2000, Smith & Kornfield 2002, Rico *et al*. 2003), however high levels of allelic diversity

require copious sampling of each population in order to insure adequate resolution.

Techniques that survey thousands of independent nuclear loci, such as Amplified

Fragment Length Polymorphisms (AFLP), have emerged as the primary tool for

elucidating the relationships between recently evolved cichlid species (Albertson *et al*.

1999, Allender *et al*. 2003, Seehausen *et al*. 2003, Schliewen & Klee 2004, Kassam *et al*.

2005, Kidd *et al*. 2006b).

AFLP is a DNA fingerprinting technique that characterizes thousands of

restriction polymorphisms spread throughout the nuclear genome (Vos *et al*. (1995).

Genomic DNA is isolated and double-digested using EcoRI and MseI restriction

enzymes. Double stranded adaptors of known sequence are then ligated onto the "sticky"

ends of the DNA fragments. An initial "preselective" PCR reaction amplifies a subset of

3

fragments that match the adapter primers and contain an additional nucleotide (EcoRI-A and MseI-C). The product of this pre-selective amplification is then used as the template for a series of different "selective" amplifications performed with primers containing any combination of 2 nucleotide extensions (eg. E-AGG, M-CTT; E-ACT, M-CTA: etc.). The fragments produced during individual "selective" PCR amplifications are denatured and separated by size on a polyacrylamide gel. Measures of genetic distance based on the binary presence or absence of AFLP fragments have been used to generate robust phylogenies for a large variety of plant and animal species (for review see Blears *et al.* 1998, Mueller & Wolfenbarger 1999). However, while early reports hailed the reproducibility of AFLP fingerprints between labs (Blears *et al.* 1998, Jones *et al.* 1998, Mueller & Wolfenbarger 1999), more recent studies question the scalability of this process (Genner & Turner 2005, Kidd unpublished data).

The patterns of divergence revealed by the AFLP based phylogenies of Albertson *et al.* (1999) encouraged Danley and Kocher (2001) to suggest that natural and sexual selection have acted in a sequential pattern of three stages during the diversification of the Lake Malawi cichlid flock. The initial stage of radiation was triggered by the adaptation of a generalized riverine ancestor to distinct macrohabitats, which produced the major lineages in the lake (the rock dwelling *mbuna* and the sand dwelling *utaka* and *chisawasawa*). The second stage is characterized by competition for trophic resources within each lineage and produced the currently recognized genera that are commonly defined by morphological characters linked to trophic specialization (Regan 1922, Trewavas 1935, Eccles & Trewavas 1989). The third stage of radiation is characterized by the divergence of secondary sexual characteristic without dramatic changes in trophic

4

morphology (Danley & Kocher 2001). Closely related species of rock dwelling mbuna often differ in color pattern (Allender *et al.* 2003), which has been shown to be an important component of conspecific mate recognition (Seehausen & van Alphen 1998, Kidd *et al.* in press). Within the sand dwelling clade, males construct elaborate species specific bowers of sand and gravel from which they display to potential mates (McKaye 1991, McKaye *et al.* 2001). While this radiation-in-stages model was initially proposed to explain diversification of the *mbuna*, it has been extended to explain species diversity in Lake Tanganyika (Salzburger & Meyer 2004) and other well-characterized vertebrate radiations (Streelman & Danley 2003). The goal of this dissertation was to test the assumptions and predictions of this and other models of cichlid speciation through a series of independent studies that evaluate behavioral, ecological and phylogenetic aspects of speciation.

Chapter 1: A Direct Assay of Female Choice in Cichlids: All the Eggs in One Basket.
The first chapter tests the critical assumption of many cichlid speciation models: that female cichlids use visual cues to recognize conspecifics. While previous attempts to quantify cichlid mate choice have yielded conflicting results (Danley 2001, Couldridge & Alexander 2001, Couldridge & Alexander 2002, Jordan *et al.* 2003), I utilize a novel testing apparatus that affords the researcher maximum control over the testing environment, but allows for the scoring of actual spawning events. With this assay I examine whether or not female *Metriaclima zebra* and *M. benetos* are able to recognize conspecific males using only visual cues.

5

Chapter 2: Intrasexual Recognition Among Closely Related Lake Malawi Cichlids: The Bars Make the Man.

Although most models of mbuna speciation focus on the effects of intersexual selection, Kornfield & Smith (2000) suggest that the extraordinary diversity of color pattern observed among male mbuna could be the result of intrasexual selection during competition for breeding territories. Recently Seehausen & Schluter (2004) proposed that intense intrasexual competition among individuals with similar color patterns could facilitate the invasion of a novel color pattern within a population, which could in turn lead to sympatric speciation. In this chapter I empirically test the fundamental supposition of Seehausen and Schluter's (2004) model: that male aggression is primarily directed toward males of a common color. Using a novel assay of male aggression designed to take advantage of cave spawning behavior of Lake Malawi mbuna, I further test the underlying assumption that male cichlids are able to recognize conspecifics utilizing visual cues. Finally, I address the question of whether the proposed reduction in aggression observed during heteromorphic interactions is the result of an inherent reaction towards a novel stimulus (as assumed by the Seehausen and Schluter model), or the product of a reinforcement-like process that develops after secondary contact between two heteromorphic species.

Chapter 3: Spatial and Temporal Dynamics of a Community of Translocated Cichlids around Thumbi West Island in Lake Malawi.

During the 1960's and 70's, an ornamental fish exporter captured numerous cichlid species endemic to northern Lake Malawi and introduced them to the already species rich

6

rocky shoreline of Thumbi West Island within Lake Malawi National Park (Ribbink *et al.* 1983, Trendall 1988). The simultaneous translocation of 17 species presents a unique opportunity to examine the role of ecology in shaping complex communities of rock dwelling cichlids. Transect data collected during the summer of 2001 was compared to previous surveys performed in 1980 and 1983 in order to evaluate the stability of this community and identify intrinsic and extrinsic factors affecting species dispersal.

Chapter 4: Patterns of Diversificaton within Pseudotropheus tropheops, a Subgenus of Rock Dwelling Cichlid from Lake Malawi.

In order to evaluate the predictions of three competing models of cichlid speciation I use a genomic fingerprinting technique called Amplified Fragment Length Polymorphisms (AFLP) to reconstruct the evolutionary history of a ubiquitous lineage of rock dwelling cichlid along the southeast arm of Lake Malawi. Species within the mbuna subgenus *Pseudotropheus tropheops* are among the most abundant cichlids observed at any rocky habitat (Fryer 1959, Ribbink *et al.* 1983) and have the widest distribution of any mbuna around Lake Malawi (Reinthal 1993). Using this phylogeny I examine the complex evolution of male color pattern, which is thought to be critical to conspecific mate recognition (Knight *et al.* 1998, Couldridge & Alexander 2002, Jordan *et al.* 2003). Using landmark-based morphometrics (Albertson & Kocher 2001), I further examine the evolution of jaw shape and habitat preference in order to assess ecological differentiation between pairs of recently evolved sister taxa.

7

Chapter 5: Axes of Differentiation in the Bower Building Cichlids of Lake Malawi.

While research and conservation efforts have historically focused on the brightly colored rock dwelling mbuna, the sand dwelling cichlids of the Lake Malawi account for 50% of the lake's biodiversity (Lewis 1982, Turner 1996). This incredibly diverse lineage affords us the opportunity to make sister group comparisons, which are one of the best ways to identify the factors involved in the evolution of diversity (Barraclough *et al.* 1998). In this chapter I use AFLP markers to reconstruct the evolutionary history of three bower building genera: *Copadichromis*, *Protomelas* and *Lethrinops* (including *Tramitichromis*). This independent molecular hypothesis was used to elucidate patterns in the evolution of bower shape and to evaluate instances of convergent evolution in morphological characters commonly used to delimit genera in morphology-based taxonomy. The patterns of divergence revealed by this phylogeny are used to evaluate the predictions of Danley & Kocher's (2001) Three Stage Model of cichlid speciation.

8

CHAPTER 1

A DIRECT ASSAY OF FEMALE CHOICE IN CICHLID FISHES: ALL THE EGGS IN ONE BASKET

Abstract

A novel testing apparatus is presented which affords the researcher maximum control over the testing environment, but allows for the scoring of actual spawning events. Female *Metriaclima zebra* and *M. benetos* chose the appropriate conspecific mate in every mating trial performed. This apparatus provides support for a critical assumption of many cichlid speciation models: that female cichlids use visual cues to recognize conspecifics. These results demonstrate that females are able to identify conspecific mates using only visual cues, and provide further support for the importance of sexual selection in the speciation of these fishes.

Introduction

Assessment of mate recognition, especially as it applies to female choice, is critical to our understanding of speciation. Behavioural isolation is often an important barrier restricting gene flow between sympatric species (Dobzhansky, 1940; Kirkpatrick & Ravigne, 2002; Ptacek 2000). Divergence of mate recognition systems is a characteristic common to many species-rich lineages (Boughman, 2002; Streelman & Danley, 2003). While Coyne and Orr (2004) caution that current barriers to gene flow may not have been the original cause of population divergence, identifying the specific traits used in species discrimination is a necessary prerequisite to identifying the forces involved in the evolution of reproductive isolation. Enumerating the traits used during conspecific mate recognition is difficult (Halliday, 1983) and requires robust assays of female choice.

Barlow (2002) identified three classes of experiments used to quantify female mate choice in fishes: free access, female only access, and restrained access. In each case females are presented with two or more males that vary for some trait (two-stimulus test, Wagner, 1998). During free access experiments (referred to as "free contact" by Turner *et al.*, 2001) all subjects are allowed to directly interact with each other. This is the most "natural" type of setting and most field observations and manipulations would fall into this experimental class. Female only access (partial partition method; Turner *et al.*, 2001) can be used when the female is smaller than the male. Size selective barriers limit male/male interaction, but still allow the female unfettered access to multiple males. In restrained access (closed chamber; Turner *et al.*, 2001) experimental subjects are confined behind transparent barriers, which limits the cues available to the female.

While female choice has been effectively quantified using each methodology, they each have specific disadvantages. Both free access and female-only access methods allow for the most

10

direct measure of mate choice (spawning, number of eggs), but their use as a research tool is limited because they do not control the types of cues available to the female (auditory, olfactory, visual, mechanical). Additionally, costly genotyping of the embryos is necessary if spawning is not witnessed (Kellogg, 1997; Knight *et al.*, 1998). In contrast, restrained access experiments offer maximum control over the testing parameters because the test subjects are isolated in individual compartments. Under these conditions the investigator is able to prevent male/male interaction and to manipulate the types of cues available to each subject. However, the physical barriers between test subjects typically force the investigator to use indirect measures to quantify mate choice. Due to its expedience, time spent in association with a subject is often used as a measure of mate choice (Forsgren, 1992; Ptacek, 1998; Brook & Endler, 2001; Gabor & Ryan, 2001; Ludlow *et al.*, 2001; Morris *et al.*, 2003; Dosen & Montgomerie, 2004; Wong *et al.*, 2004). Unfortunately, using an indirect measure can lead to the misinterpretation of female choice when the organism's motivational state is not considered (Barlow, 1989). Specifically, fear response (Barlow, 2002), the female's reproductive state (Clement *et al.*, 2005) and aggression (Danley, 2001) can obscure female choice. Clement *et al.* (2005) found that while gravid female *Haplochromis burtoni* (Günther) tend to associate with territorial males, non-gravid females show no preference. The use of indirect measures of mate choice can lead to errors of interpretation when the organism's natural history is not carefully considered (Scott & Foster, 2000).

The cichlid fishes of East Africa make an excellent model for studying the role of mate choice in speciation. Intersexual selection has likely played a key role in the explosive speciation of Lake Malawi's 500+ species of haplochromine cichlids (Dominey, 1984). Rock dwelling cichlids (*mbuna*) of Lake Malawi are often distinguished on the basis of male colour pattern and

11

frequently show strong sexual dimorphism (Fryer & Iles, 1972; Ribbink *et al.*, 1983; Deutsch, 1997; Stauffer *et al.*, 1997; Turner *et al.*, 2001). Even though several congeners are typically found together in each mbuna community, few hybrids have been observed in the wild. Interspecific and even intergeneric fertile hybrids are easily produced in the lab by restricting female choice (Crapon de Caprona, 1984; Albertson *et al.*, 2003), suggesting that pre-mating behaviours maintain reproductive isolation. The importance of behavioural isolation and the large variance in reproductive success among males on a lek, suggest that female choice may be an important mechanism of selection (Hert, 1989; Deutsch, 1997; Seehausen *et al.*, 1999). Several recent models propose that intersexual selection on male colour pattern has played an important role in speciation (Turner & Burrows, 1995; Deutsch, 1997; van Doorn *et al.*, 1998; Higashi *et al.*, 1999; Kondrashov & Kondrashov, 1999; Seehausen *et al.*, 1999; Takimoto *et al.*, 2000; Lande *et al.*, 2001).

Models that depend on female choice to initiate divergence are predicated on the assumption that females recognize conspecifics using visual cues. Unfortunately, attempts to quantify female choice in cichlids have produced conflicting results. Orientation toward, or time spent in proximity to the subject is often used as a measure of cichlid mate choice (Falter & Charlier, 1989; Beeching & Hopp, 1999; Couldridge & Alexander, 2001; Couldridge & Alexander, 2002; Jordan *et al.*, 2003; Werner & Loten, 2003). Couldridge & Alexander (2001) suggested that time spent could be used to quantify mate choice in a restricted access experiment of cave spawning *Metriaclima zebra* (Boulenger) 'red dorsal'. However, the effectiveness of 'time spent' was corroborated by an unnatural 'pit digging' behaviour. Seehausen *et al.* (1998) found that scoring "relative responsiveness" in a restrained access assay yielded indeterminate results with a population of *Pundamilia nyererei* (Witte-Maas & Witte) that demonstrated completely

12

assortative mating during free access experiments. Additionally, Danley (2001) found that female *Metriaclima benetos* (Stauffer, Bowers, Kellogg & McKaye) were more likely to associate with heterospecific *M. zebra* males, possibly because of an unusual aggressive response toward heterospecific males. Measuring time spent by a female cichlid may be analogous to scoring "visiting" behaviour in the field, which often includes heterospecific visits (Seehausen *et al.*, 1998) and is poor indicator of spawning success (McKaye *et al.*, 1990; Kellogg *et al.*, 2000).

In this study, a novel experimental technique is used to quantify female mate choice among members of the *Metriaclima zebra* complex from Lake Malawi. This "egg catcher" design uses a restrained access assay to eliminate male/male interactions, which can inhibit female choice (Morris *et al.*, 1992; Kodric-Brown, 1993). Instead of quantifying female choice with an indirect measure, an artificial spawning cave with a false bottom was used to collect eggs as they were released. This method reduces the ambiguity of female motivation. While this assay is used to assess mate choice in cichlid fishes, it may be readily adaptable to a variety of substrate (ex. anemonefish, killifish, pupfish) and nest spawning (ex. centrarchids, gobies, sticklebacks) species of fish.

13

Methods and Materials

Study Animals

Species of the cichlid genus *Metriaclima* live in rocky habitats throughout Lake Malawi, Africa. While *Metriaclima zebra* is found at many localities around the lake, *M. benetos* is endemic to Mazinzi Reef (Stauffer *et al.*, 1997). On this reef, the two species co-exist on similar habitats and their territories often interdigitate. *Metriaclima zebra* and *M. benetos* are ecologically, morphologically, and behaviourally similar, but can be distinguished easily on the basis of adult colour pattern.

Territorial *M. zebra* males are bright blue with 5-7 black vertical body bars, a black bar in their pelvic fin, and display a prominent black interorbital bar (Fig. 1b). Territorial *M. benetos* are also bright blue, but lack the conspicuous melanistic markings observed in *M. zebra* (Fig. 1a). Females of both species are drab olive brown (Fig 1). Female *M. zebra* also exhibit many of the same melanistic patterns observed in male *M. zebra*; however, these markings are much less conspicuous in the females than territorial males. Morphologically, *M. zebra* and *M. benetos* exhibit minor differences in horizontal eye diameter, caudal peduncle length and number of dorsal spines (Stauffer *et al.*, 1997). The animals used in these studies were first and second generation offspring of wild-caught animals collected at Mazinzi Reef in the southeast arm of Lake Malawi.

Spawning behaviour is stereotyped and appears to be identical among mbuna species (McElroy & Kornfield, 1990). Most mbuna cichlids are promiscuous. Females often visit and spawn with several males (Kellogg *et al.*, 1995; Parker & Kornfield, 1996). Male mbuna compete for permanent territories, from which they solicit females through a pattern of courtship

14

displays. When a female approaches a territorial male, he will respond with a series of lateral displays and quivers. Males will then lead the female toward the location within his territory used for spawning (cave, flat rock surface, etc.). If the female follows, they enter the spawning area where she will deposit her eggs and immediately circle to pick them up into her mouth. During spawning the female nudges the male's anal fin and he releases sperm to fertilize the eggs held within her buccal cavity (Fryer & Iles, 1972; Holzberg, 1978; McElroy and Kornfield, 1990).

Egg-Catcher Assay

The experimental design was modified from Nelson (1995). Males were housed in separate, but adjacent 37.8l aquariums (Fig. 2). The test female was placed in a 193.5l aquarium adjacent to the males. While the female had visual access to both males, the males were unable to see one another, preventing male-male interactions. Clay flowerpots were cut in half and glued to the aquarium walls to construct spawning caves, which allowed males and females to remain in visual contact during spawning. The bottom of the cave was constructed of plastic grating with $1 cm^2$ openings spaced 1mm apart. A dish was placed under the grating to collect any eggs laid during spawning. The combination of grating and dish prevented the female from picking the eggs up in her mouth, so that the number of eggs laid with each male could be accurately determined.

Males were matched for size (difference in standard length less than 2mm) and randomly assigned to stimulus tanks. Males were allowed to acclimatize for 48 hours prior to the introduction of the female. A female *M. zebra* or *M. benetos* was introduced to a neutral shelter

15

in the center of the testing aquarium in the morning and left until eggs were deposited.

Receptacles were checked twice daily for eggs, and each trial continued until eggs were found.

The presence of eggs was considered to be an indication of the female's choice of a specific

male. After the trial, the female was removed and the testing chamber was drained and bleached

to remove any pheromonal cues, which might promote female copying behaviour. The next pair

of heterospecific males were arranged in alternate stimulus tanks to avoid side bias. Sixteen

males of each species were used to test female preference. Due to a shortage of stock, four males

of each species were reused, but were never paired with the same heterospecific male. The

females used in these trials had no previous contact with the any of the specific males presented,

however they were raised with other conspecifics. Ten females of each species were tested. The

number of observed spawns each male received was compared to random mating using Fisher's

Exact Test.

16

Results

Trials ran a mean of 33.8 days ($\overline{X}\pm$SE=35.3$\pm$8.84 for *M. benetos* and $\overline{X}\pm$SE=32.0$\pm$10.42 for *M. zebra*), which is similar to the duration of a female only access trial with the same species ($\overline{X}$=25 days, range=8-44 days, Kellogg, 1997) and with *Astatotilapia elegans* (32-35 days, Hert, 1989). Eggs were never found in more than one dish during any of the trials, and no heterospecific spawning events were observed (Table I). Females deposited eggs in the cave nearest the conspecific male in all 20 trials (10 for each species). These results demonstrate complete assortative mating (Fishers's Exact Test, p=0.039 for both species).

Occasionally, a female was found holding some of the eggs in her buccal cavity. Females were never observed to be holding eggs, unless some eggs had also been deposited in a dish. However, since we could not discount the possibility that mouthbrooded eggs had been spawned elsewhere in the testing arena, these eggs were not scored. The mean number of eggs recovered from each species was nearly identical ($\overline{X}\pm$SE= 20.25$\pm$7.17 for *M. benetos*, $\overline{X}\pm$SE=19.88$\pm$5.74 for *M. zebra*) with a total range of 2-70 eggs/spawn (3-70 for *M. benetos* and 2-61 for *M. zebra*). This is very similar to the brood sizes reported for other populations of *M. zebra* in female only access trials ($\overline{X}$=20.6 embryos, range=2-56 embryos, Knight & Turner, 2004) and *Astatotilapia elegans* ($\overline{X}\pm$SD=21.8$\pm$7.8 embryos, range not reported, Hert, 1989). The size of broods collected in the "egg catcher" apparatus was also similar to those observed in captive ($\overline{X}\pm$SE =24.5$\pm$11.5 embryos, range not reported, Holzberg, 1978) and wild populations of *M. zebra* ($\overline{X}\pm$SD =26.0$\pm$6.7 embryos, range not reported, Marsh *et al.*, 1986).

Discussion

Attempts to quantify mate choice often rely on indirect measures during restrained access experiments where male and female fish are physically separated (Wagner, 1998). While physical barriers between test subjects are necessary to control the cues used by the individuals making a choice, it also forces the investigator to use indirect measures to quantify that choice. Studies in guppies (Kodric-Brown, 1993), sand gobies (Forsgren, 1992), and Pacific blue-eyes (Wong, 2004) have demonstrated that association time can be an effective measure of eventual copulation success. However, all of these studies evaluated intraspecific variation as opposed to conspecific recognition. Gabor (1999) has demonstrated that female sailfin mollies (*Poecilia latipinna*) may associate with larger individuals for reasons other than mate choice. In several species (sticklebacks; Hay & McPhail, 1975; darters; Fuller, 2003; and cichlids; Seehausen, 1997; Danley 2001) association time fails to demonstrate the assortative mating observed in the wild or during free access experiments. It may be misleading to equate time spent with mate choice, without corroboration of some subsequent spawning behaviour or confirmation of the subject's reproductive state.

Barlow (2002) suggested that the inclusion of specific courtship behaviours would help to distinguish mating activity from a fear response. The use of unambiguous spawning behaviour has been used effectively to quantify mate preference in several species of fish (Nagel & Schluter, 1998; Amundsen & Forsgren, 2003; Fuller 2003). Unfortunately, the typical courtship behaviour of a haplochromine female is to follow and circle with the displaying male (Fryer & Iles, 1972; Holzberg, 1978; McElroy and Kornfield, 1990), which is difficult to score in a restricted access assay. Behaviours such as quivering, lateral displays, biting, head shaking, and approaching have been used to quantify female mate preference (Falter & Charlier, 1989; Barlow

18

& Sir, 1997; Seehausen & van Alphen, 1998; Jordan *et al.*, 2003). Many of these behaviours are used in both courtship and aggression (Baerends & Baerends van Roon, 1950), so it is sometimes difficult to distinguish the motivation driving the individual's behaviour (Danley, 2001). The motivational uncertainty inherent in indirect behavioural measures compels us to use direct measures of mate choice whenever possible. The 'egg catcher' assay combines the control of a 'restrained access' experiment with the power of a direct measure of mate choice.

The egg catcher assay unambiguously demonstrated that conspecific mate choice is possible when only visual cues are available to the female. To our knowledge, this is the first study to use a direct measure of conspecific mate recognition using only visual cues in cichlid fish. Previous studies have demonstrated that species (Kellogg, 1997; Knight *et al.*, 1998; van Oppen *et al.*, 1998) and even populations (Knight & Turner, 2004) of mbuna mate assortatively when all cues are available. Further Couldridge & Alexander (2002) and Jordan *et al.* (2003) found that females tend to associate with conspecifics when presented with visual cues alone. Seehausen *et al.* (1997) suggested that visual cues are so important to conspecific recognition that a change in the normal photic environment results in the loss of species cohesiveness. Seehausen & van Alphen (1998) were able to disrupt conmorphic mate choice between the blue and red morphs of *Pundamilia nyererei* when females viewed males under monochromatic light. In a similar experiment female *Metriaclima zebra* (blue barred), *M. benetos* (solid blue) and *M. barlowi* (gold body with blue fins) were still able to recognize conspecifics under monochromatic light (Jordan *et al.*, 2003). While both *P. nyererei* morphs share a common melanistic pattern, the three *Metriaclima* species display differing patterns of colour contrast, which would not have been obscured by the change in lighting.

19

While this study demonstrates that visual cues are sufficient for mate choice, it does not preclude the possibility that multiple signals or sensory modalities are utilized during mate assessment (Hankison & Morris, 2003). A hierarchy of cues may be used during intraspecific mate choice. Females could be basing their choice on differences in male colour pattern, morphology, behaviour repertoire, or some subset/combination of each. Genus specific differences in courtship "dances" have been reported for sympatric species of sand-dwelling haplochromine cichlids (Stauffer *et al.*, 1993), but McElroy & Kornfield (1990) failed to see any species-specific courtship behaviours among mbuna cichlids. Specific differences in the temporal elements of courtship sounds have been identified between sympatric genera of Lake Malawi sand-dwellers (Lobel, 1998) and species of mbuna (Amorim *et al.*, 2004). While there is sexual dimorphism in the enzyme activity of muscles that tend to be associated with sound production (Rice & Lobel, 2002), the effect of these auditory signals on mate choice remains to be tested. The use of olfactory cues have been demonstrated in other cichlid species (Chien, 1973; Capron de Caprona, 1980), but Jordan *et al.* (2003) reported that female mbuna failed to respond differently when exposed to water containing conspecific versus heterospecific olfactory cues. The 'egg-catcher' design allows new approaches to identify both the specific cues used in inter- and intraspecific mate choice and the synergistic effects of various signal modalities.

The complete assortative mating demonstrated in this experiment could also be consistent with male choice of the females based on visual cues. If males are able to recognize conspecific females, then they may be selectively displaying to conspecific females. Knight & Turner (1999) found that male members of the genus *Metriaclima* were able to differentiate between hetero- and conspecific females only when the female colour patterns were different (e.g. OB vs. barred). If female colour patterns were similar, or identical, then the males were unable to

20

distinguish hetero- from conspecifics. Seehausen & van Alphen (1998) found that while female *Pundamilia nyererei* displayed a preference for conspecific males, female colour form had no effect on male courtship. This is consistent with the observation of males displaying to heterospecific females during this study. Assessment of male courtship behaviours during the initial introduction of the female could be used to delineate between male and female choice (Nelson, 1995).

Female preference for male colour patterns is a necessary prerequisite for many models of cichlid speciation via female mate choice (Turner & Burrows, 1995; Deutsch, 1997; van Doorn *et al.*, 1998; Higashi *et al.*, 1999; Kondrashov & Kondrashov, 1999; Seehausen *et al.*, 1999; Takimoto *et al.*, 2000; Lande *et al.*, 2001). If this pattern of assortative mating by visual cues is consistent among the mbuna, then these models could provide a mechanism for the origin and maintenance of the incredible diversity of male colour patterns found within Lake Malawi.

21

Table 1.1: Results of the Egg Catcher Assay.
Females were presented with a simultaneous choice between a heterospecific and conspecific male. Male reproductive success was indicated by the presence of eggs within his territory. Using only visual cues, females demonstrated complete assortative mate choice by depositing eggs in the caves of conspecifics (Fisher's Exact Test, p<.05).

Female	Number of Spawns with Male		Number of Eggs Deposited with Male	
	M. zebra	M. benetos	M. zebra	M. benetos
M. zebra	10	0	152	0
M. benetos	0	10	0	166

22

Figure 1.1

Color patterns of *Metriaclima benetos* (a) and *Metriaclima zebra* (b). While territorial males (a1, b1) of both species possess a blue background, only *M. zebra* displays melanistic patterns, which include 5-7 black vertical bars, a black bar on the pelvic fins, and a black "mask." Females (a2, b2) of both species are a drab olive/brown color with a pattern of weak vertical bars.

23
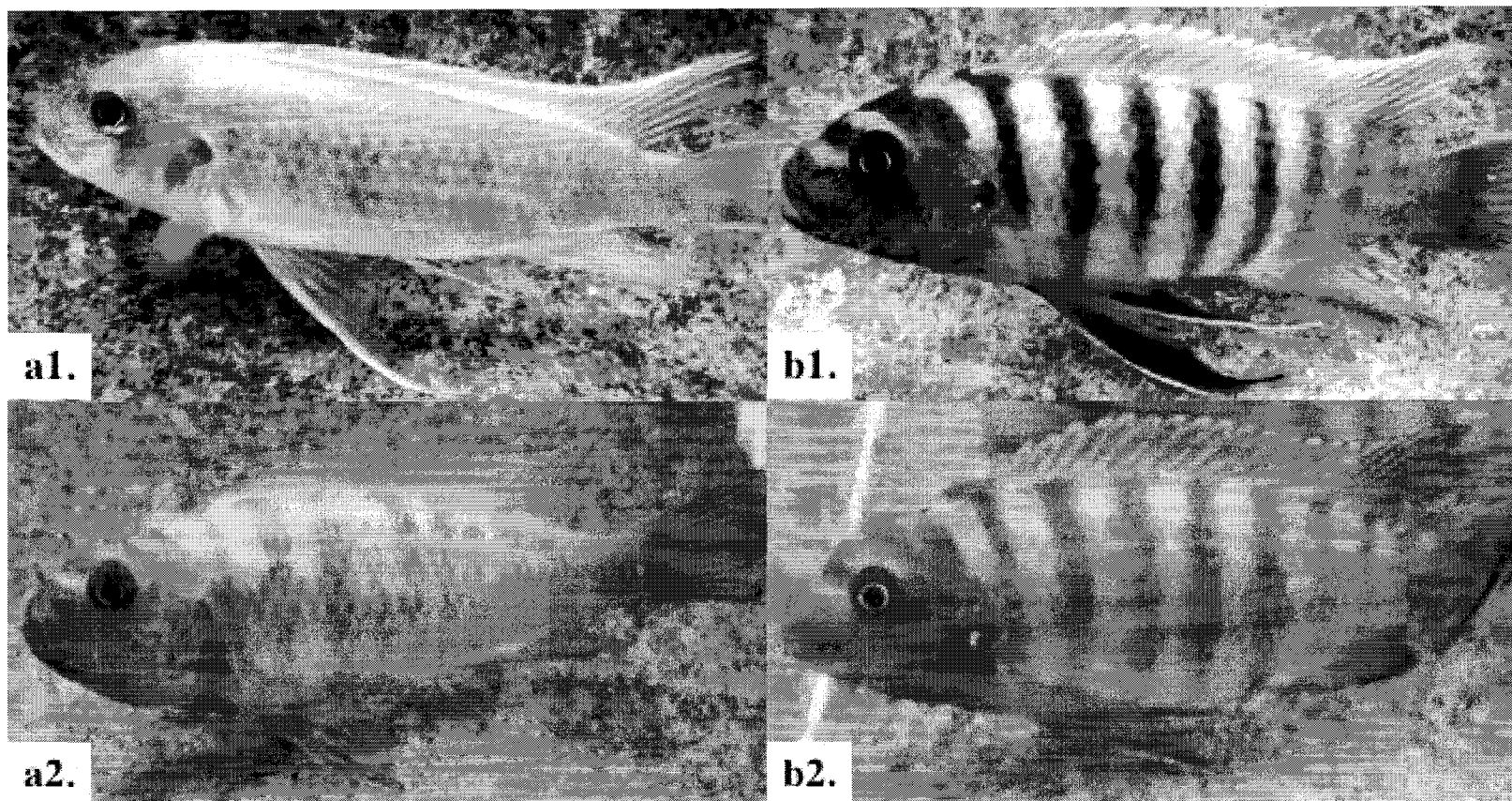
Figure 1.2
Test arena for the egg catcher assay of mate choice (top and front view). While, double pane glass prevented auditory, tactile, and olfactory communication between males and females, the split caves allowed visual interaction between male and female subjects throughout the spawning sequence. Eggs laid within the cave fell through the slit cave floor and into the egg receptacle. Male success was scored as the presence on eggs in the cave within his territory.
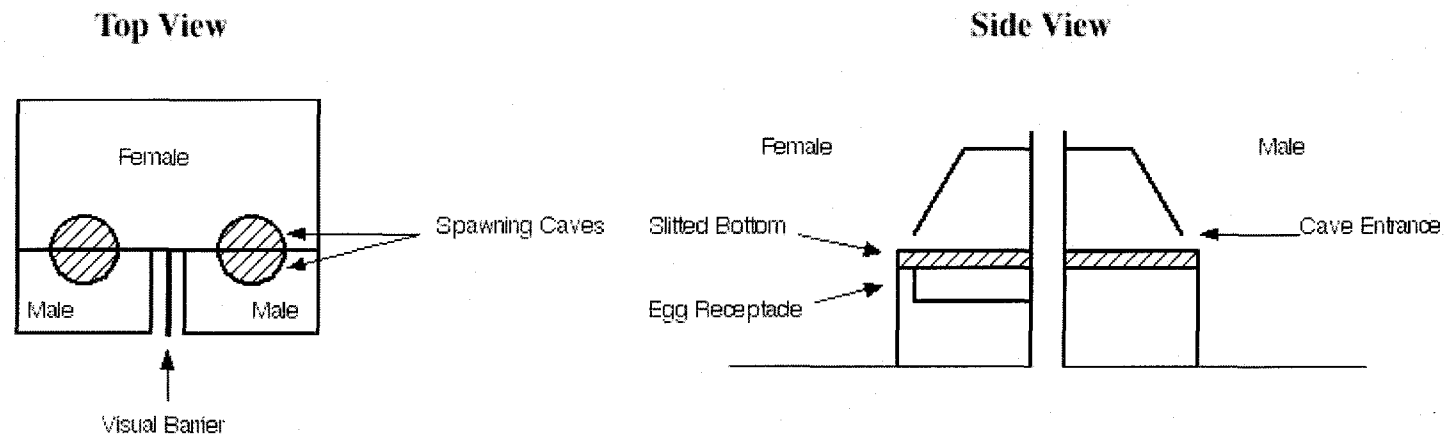
CHAPTER 2

INTRASEXUAL RECOGNITION AMONG CLOSELY RELATED LAKE MALAWI CICHLIDS: THE BARS MAKE THE MAN

Abstract

While intersexual selection is widely assumed to have played a key role during the

explosive radiation of East African haplochromine cichlid fishes, a recent model

proposed that intense intrasexual competition among individuals with similar color

patterns could facilitate the invasion of a novel color pattern within a population, which

could in turn lead to sympatric speciation (Seehausen & Schluter 2004). I tested the

assumptions of this model by comparing aggressive behavior during territorial contests

among five closely related cichlid species from Lake Malawi, Africa. I found that

interactions between conspicuously heteromorphic males tend to be less aggressive than

those between those of homomorphic males, but territorial contests between similarly

colored heterospecifics are as intense as interactions between conspecifics. Further, the

reduced aggression displayed by *Metriaclima zebra* toward a geographically and

phylogenetically unique color pattern suggests that this pattern of behavior is the inherent

response to a novel stimulus, rather than the product of an evolutionary process that

occurs after secondary contact between heteromorphic species. While intrasexual

competition has likely influenced the evolution of conspicuous differences in male

25

coloration, it is probably not sufficient to explain more subtle differences observed

among Lake Malawi cichlids.

26

Introduction

The role of sexual selection during the evolution of male ornamentation has been well studied (Anderson 1994, Kirkpatrick 1987). Over a century has passed since Darwin suggested that sexual selection could foster the formation of new species (Darwin 1871), however its role in speciation remains highly contested. Divergence of mate recognition systems can be a powerful force generating prezygotic isolation between populations with or without secondary ecological divergence (Panhuis *et al.* 2001, Boughman 2002, Kirkpatrick & Ravigné 2002). While several models suggest that sexual selection has the potential to drive speciation (Wu 1985, Turner & Burrows 1995, Higashi *et al.* 1999, Takimoto *et al.* 2000), they often require a complex set of assumptions that may not be biologically relevant (Kirkpatrick & Ravigné 2002). Unfortunately, testing the parameters of these models is often difficult because of the long time scales over which new species typically form.

The monophyletic assemblage of 700+ cichlid species in Lake Malawi represents a unique opportunity to study the role of animal communication during vertebrate speciation (Kornfield & Smith 2000, Stauffer *et al.* 2002, Genner & Turner 2005). While Lake Malawi is estimated to have formed 2-5 million years ago (MYA) (Salzburger & Meyer 2004), recent molecular evidence suggests that much of the current cichlid fauna formed within the last 10,000 years (Won *et al.* 2005). The brightly colored rock-dwelling species (locally known as mbuna) have long attracted the attention of evolutionary biologists (Fryer & Iles 1972). At any location it is common to find multiple congeners with overlapping trophic utilization but differing male color pattern (Ribbink

27

et al. 1983, Konings 2001). Molecular phylogenies suggest that particular color patterns have evolved repeatedly within genera (Smith & Kornfield 2002, Allender *et al*. 2003, Kidd in prep.). Intersexual selection has often been invoked to explain the incredible diversity of male color pattern observed among Lake Malawi's rock dwelling cichlids (Dominey 1984, Seehausen *et al*. 1996, Deutsch 1997, Danley & Kocher 2001, Turner 2001, Genner & Turner 2005).

The discriminating mate preferences displayed by female cichlids in captivity inspired Kosswig (1947) to suggest that selective mating was a critical factor contributing to the accelerated speciation of East African cichlids. Territorial mbuna males are arranged in breeding arenas, or leks, centered on a spawning substrate (e.g. a cave). Females swim through these leks and breed with multiple males (Parker & Kornfield 1996). After spawning, females leave the breeding arena and assume sole responsibility for parental care. The importance of male color pattern on female mate recognition is well documented (Knight *et al*. 1998, Couldridge & Alexander 2002, Jordan *et al*. 2003, Kidd *et al*. 2006a). Female mbuna appear to be able to identify conspecific males when visual cues alone are available (Kidd *et al*. 2006a). It has been further suggested that sexual selection via female choice has been the dominant mechanism of differentiation during recent cladogenic events (Danley & Kocher 2001, Streelman & Danley 2003).

Although most models of mbuna speciation focus on the effects of intersexual selection, Kornfield & Smith (2000) suggest that the extraordinary diversity of color pattern observed among male mbuna could be the result of intrasexual selection during competition for breeding territories. Competition for territories is critical to the ability of an individual male to reproduce (Fryer & Iles 1972, Genner & Turner 2005). Only about

28

half of the adult males in a population occupy a territory (Holzberg 1978, Hert 1990, but see Genner *et al.* 1999), which they may hold for over six months (Hert 1995). Non-territorial males generally exhibit color patterns similar to juveniles and females and do not achieve full coloration until they have gained a territory (Ribbink *et al.* 1983, Genner & Turner 2005). Sympatric congeners within the same lek form a mosaic of overlapping territories. While heterospecific territories may overlap, conspecific territories rarely do (Holzberg 1978, Hert 1990, Genner et al. 1999, Danley 2001). Removal experiments reveal that conspecific males swiftly reoccupy territories (Hert 1990, Danley 2001), suggesting that while intraspecific competition for territories is intense, interspecific competition is quite low. While some studies of sand dweller breeding arenas have failed to find female preference for specific male characteristics, they often find that female's exhibit preference for central positions within the lek, which are gained through intrasexual contests (McKaye 1991, Kellogg *et al.* 2000).

Seehausen and Schluter (2004) proposed that intense male-male competition would facilitate the invasion of a novel male color form within a population. If aggression is primarily directed toward males of a common color, then individuals expressing a novel color pattern will be able to acquire and defend a territory more easily. They further suggest that if female choice were based on a conglomerate of cues (eg. territory quality, increased vigor) and not just male color pattern, then the frequency of the novel male phenotype would increase within the population even in the presence of opposing female preference. As female preference for the invading color form increases through a Fisherian-like process then two outcomes are possible: a stable male polymorphism, or two reproductively isolated color morphs. Based on this model

29

Seehausen and Schluter (2004) propose that intrasexual selection can trigger speciation when a novel color form occurs within a monomorphic population (through mutation, hybridization, or recombination), or when allopatric populations with different rare male color polymorphisms come into secondary contact (Seehausen & Schluter 2004). Searching for the expected fingerprint of character displacement among rock dwelling Lake Victorian cichlids, Seehausen and Schluter (2004) found that the local occurrence of species is negatively correlated with those of closely related species of similar color. Conversely, local occurrence is positively correlated with closely related species of different colors.

Our study empirically tests the fundamental supposition of Seehausen and Schluter's (2004) model: that male aggression is primarily directed toward males of a common color. If this assumption is accurate then the reverse hypothesis should also be true: that heterospecific interactions between males of homomorphic species should be as intense as conspecific interactions. Using a novel assay of male aggression designed to take advantage of the cave spawning behavior of Lake Malawi mbuna, I further test the underlying assumption that male cichlids are able to recognize conspecifics utilizing visual cues. Finally, I address the question of whether the proposed reduction in aggression observed during heteromorphic interactions is the result of an inherent reaction towards a novel stimulus (as assumed by the Seehausen and Schluter model), or if it is the product of a reinforcement-like process that develops after secondary contact between two heteromorphic species.

Study Species

Metriaclima is one of the most species rich genera of mbuna within Lake Malawi (Ribbink *et al.* 1983, Stauffer *et al.* 1997, Konings 2001). While some species in this group have wide distributions, others show a high degree of endemism. It is common to find 3-6 congeners with overlapping territories at any given locality (Ribbink *et al.* 1983, Koning 2001). Recent molecular evidence indicates that the genus *Metriaclima* is composed of distinct northern and southern clades (Allender *et al.* 2003). Within each clade particular color patterns have evolved in parallel (Smith & Kornfield 2002, Allender *et al.* 2003, Kidd *et al.* in prep.). Non-territorial males typically exhibit juvenile coloration and school with females foraging in the water column. These males frequently swim through the lek, occasionally sparring with territorial males (Holzberg 1978, Schröder 1980).

Intraterritorial contests are highly stereotyped within the family Cichlidae and begin when a subdominant male enters the territory of a conspecific (Baerends & Baerends-van Roon 1950, Hurd 1997, Barlow 2000). The territorial male responds by displaying its median fins erect while orienting perpendicularly towards the intruder (lateral display). The behavior of the resident male often intensifies to include a gular flare (extension of the branchiostegal membrane) and shivering of the entire body. Continuing all display behaviors, the two males will then circle each other and move into a parallel head to tail position. This position often leads to a tail beat, but can also act as a springboard for a quick turn and bite of the opponent's flank or tail. Intraterritorial contests usually end with the withdrawal of the intruding male while being chased by the

resident (Holzberg 1978, McKaye 1984, Hert 1990). These contests typically occur between conspecifics and are more complex and stereotyped than the simple 'chase and withdrawal' of heterospecific encounters (Baerends & Baerends-van Roon 1950, Holzberg 1978, Kidd per. obs.).

Aggression Assay

In order to elicit territorial contests, an assay was designed that takes advantage of the natural territorial behavior of cave spawning *Metriaclima* (McElroy & Kornfield 1990, Kidd *et al.* 2006a). Captive stock was raised in single species tanks prior to isolation of individual test subjects in 37.8 l aquaria for a minimum of 3 weeks prior to testing. The experimental setup consisted of a series of 4 adjacent, but separate 37.8 l aquariums with flanking removable opaque barriers (Figure 1). Test males were matched for size (difference in standard length, L_S <2mm) before being randomly assigned to one of the two center stimulus tanks. These aquaria included a clay flowerpot that had been cut in half and glued to the interior wall of each aquarium to serve as spawning caves. With all visual barriers in place, conspecific females were then introduced to the adjacent outer aquaria. The fish were allowed to acclimatize for 24 h prior to the removal of the visual barriers between male and female conspecific pairs. Males were monitored for coloration, courtship and territorial behaviors. If after 48 h, each male exhibited full territorial coloration, then the barriers between male/female pairs were replaced and the visual barrier between the subject males was removed. While the barrier was in place each male occupied an isolated territory, but after the barrier was removed the test males visually occupied the same territory focused on the same flowerpot. Double pane glass

32

prevented auditory, tactile, and olfactory communication between subjects. Male-male interactions were filmed for 20 minutes. This protocol was used to examine the role of male color pattern during territorial contests in three experiments.

Experiment 1 - Captive raised vs. wild caught *Metriaclima zebra*

This study included males of wild caught, 1st, 2nd, and 3rd generation captive bred stock (Table 1). In order to evaluate the similarity of response between wild caught and captive raised subjects, I tested 20 individual (10 trials) wild caught *M. zebra* collected from Mazinzi Reef in 2005 and compared them to 20 individual (10 trials) 2nd and 3rd generation captive bred males raised from stock collected at the same location in 1996.

Experiment 2 – Black barred vs. blue male color pattern

While species of the mbuna genus *Metriaclima* exhibit an incredible array of male color patterns, the most common are the 'black barred' (BB) and 'solid blue' (BL), which can be found throughout Lake Malawi (Ribbink *et al*. 1983, Stauffer *et al*. 1997, Konings 2001). Territorial *M. zebra* males from Mazinzi Reef are bright blue with 5-7 black vertical body bars, a black bar in their pelvic fin and a prominent black interorbital bar (Figure 3d). Found along the central eastern coast, *M. phaeos* has a color pattern very similar to *M. zebra*, but with a prominent black submarginal band in its dorsal fin as well as yellow on the ray portion of the dorsal fin and on the trailing edge of the caudal fin (Figure 3c). Territorial *M. benetos* of Mazinzi Reef (Figure 3a) and *M. callainos* of Nkhata Bay (Figure 3b) are also bright blue, but lack the conspicuous melanistic markings observed in either *M. zebra* or *M. phaeos*. While *M. callainos* is part of the northern clade, *M. benetos* represents a parallel evolution of the solid blue coloration

33

within the southern clade (Allender *et al.* 2003). *M. callainos* naturally occurs at Nkhata

Bay and at sites along the northern coast of Lake Malawi, but the stock used in this

experiment were collected from a population introduced to Nankoma Island in the 1970's

(Ribbink *et al.* 1983, Trendall 1988). Each species was tested in 10 conspecific and 30

heterospecific trials (10 for each BB and BL species). In order to ensure that paired males

were matched for size, some males of each species were used during both conspecific and

heterospecific trials (Table 1). Any males that were used more than once were reisolated

for a minimum of 4 weeks between trials.

Experiment 3 – Phylogenetically novel color pattern

Seehausen and Schluter's (2004) model assumes that reduced aggression between

heteromorphic individuals is the inherent result of exposure to a novel color pattern.

However, since many of the color patterns exhibited by territorial males are recapitulated

throughout Lake Malawi (Smith & Kornfield 2002, Allender *et al.* 2003), it is difficult to

separate this hypothesis from the possibility that reduced aggression is the result of a

process of evolutionary reinforcement after the secondary contact of two heteromorphic

species. In order to separate these alternate hypotheses I conducted 10 heterospecific

trials with *M. zebra* and *Metriaclima* 'msobo,' an undescribed species from Magunga

Reef in northeastern Lake Malawi. While essentially blue-black, *M.* 'msobo' exhibits an

irregular melanin pattern with strong horizontal elements (Figure 4), which is dissimilar

to any other species of *Metriaclima* (Konings 2001, Genner & Turner 2005). Phylogenies

inferred from Amplified Fragment Length Polymorphisms (AFLP) indicate that while *M.*

zebra from Mazinzi Reef is part of the southern clade of *Metriaclima* (Allender *et al.*

34

2003), *M.* 'msobo' is embedded within the northern clade (Kidd *et al.* in prep). *Metriaclima zebra* from Mazinzi Reef is therefore both geographically and phylogenetically naïve to this unique color pattern.

Data Analysis

Of the behaviors common to territorial contests, only lateral displays and bites were amenable to reliable quantification during these trials. Lateral displays were defined as the erection of median fins, with or without a gular flare, while the subject's body was parallel to the glass partition (Baerends & Baerends-van Roon 1950). Discrete lateral displays terminated when the fins were lowered against the body, the subject left an 'active zone' equal to one body length from the glass, or when interrupted by another behavior. Bites were defined as open mouth contact against the glass (typically forcefully) toward the opponent. The number of lateral displays and bites were recorded and comparisons between conspecific and heterospecific trials were made using analysis of variance (ANOVA) followed by Tukey's test in Systat. Square-root transformation was used on data when Levene's tests revealed unequal variances between groups. While both males were observed and scored during each conspecific trial, degrees of freedom were calculated based on 10 independent trials rather then 20 individual observations in order to avoid pseudoreplication (Barlow 1989).

<u>Results</u>

A total of 180 20min trials were videotaped and scored for the number of bites and lateral displays. Eleven additional trials were discontinued (6.1%) when males failed to achieve territorial coloration while presented with the conspecific female.

<u>Experiment 1</u>

There was no significant difference between the number of bites (ANOVA: $F_{1,18}=$ 1.067, P=0.315) or the number of lateral displays (ANOVA: $F_{1,18}=0.494$, P=0.491) performed by wild caught and captive reared male *Metriaclima zebra* during conspecific territorial contests (Figure 2). The results from the conspecific trials of captive raised and wild caught *M. zebra* were merged and used for all subsequent analysis (Figure 3 and 4).

<u>Experiment 2</u>

Males performed more bites (ANOVA: $F_{1,166}=78.316$, P<0.001) and lateral displays (ANOVA: $F_{1,166}=102.242$, P<0.001) during homomorphic territorial contests than they did then during heteromorphic interactions (Figure 3). Overall, species with solid blue coloration (BL, *M. benetos* and *M. callainos*) performed more bites (ANOVA: $F_{1,166}=138.895$, P<0.001) and lateral displays (ANOVA: $F_{1,166}=9.224$, P=0.003) than males with black barred coloration (BB, *M. phaeos* and *M. zebra*). The pattern of increased number of bites (ANOVA: $F_{1,166}=12.169$, P=0.117) and lateral displays (ANOVA: $F_{1,166}=4.454$, P=0.158) observed during homomorphic interactions was similar between both male color patterns (BL/BB).

36

Metriaclima benetos displayed significantly different numbers of bites and lateral

displays (reported respectively throughout) during territorial contests with other species

(ANOVA: $F_{1,36}$=10.316, P<0.001; ANOVA: $F_{1,36}$=15.186, P<0.001). When compared to

conspecific interactions, *M. benetos* (Figure 3a) preformed fewer bites and lateral

displays against heteromorphic *M. zebra* (Tukeys HSD df=36, p<0.001; Tukeys HSD

df=36, p<0.001) and *M. phaeos* (Tukeys HSD df=36, p=0.028; Tukeys HSD df=36,

p=0.001), but exhibited similar levels of aggression toward the homomorphic *M.*

callainos (Tukeys HSD df=36, p=0.712; Tukeys HSD df=36, p=0.999). Similarly, M.

callainos exhibited significantly different levels of aggression against heterospecifics

(ANOVA: $F_{1,36}$=7.080, P=0.001; ANOVA: $F_{1,36}$=8.927, P<0.001). While *M. callainos*

(Figure 3b) was as aggressive against *M. benetos* as it was against conspecifics (Tukeys

HSD df=36, p=0.997; Tukeys HSD df=36, p=0.994), contests against heteromorphic *M.*

phaeos (Tukeys HSD df=36, p=0.002; Tukeys HSD df=36, p=0.001) and *M. zebra*

(Tukeys HSD df=36, p=0.033; Tukeys HSD df=36, p=0.004) were significantly less

intense.

While generally less aggressive than *M. benetos* and *M. callainos* during

conspecific and heterospecific contests, both *M. phaeos* (ANOVA: $F_{1,36}$=5.961, P=0.002;

ANOVA: $F_{1,36}$=5.598, P=0.002) and *M. zebra* (ANOVA: $F_{1,36}$=6.937, P=0.001; ANOVA:

$F_{1,36}$=6.177, P=0.001) demonstrated significant differences in levels of aggression against

heterospecifics. *Metriaclima phaeos* (Figure 3c) was similarly aggressive during

conspecific and heterospecific intraterritorial contests with *M. zebra* (Tukeys HSD df=36,

p=0.762; Tukeys HSD df=36, p=0.966), but performed fewer aggressive acts toward *M.*

callainos (Tukeys HSD df=36, p=0.007; Tukeys HSD df=36, p=0.011) and *M. benetos*

37

opponents (Tukeys HSD df=36, p=0.009; Tukeys HSD df=36, p=0.016). While *M. zebra* performed as many aggressive acts toward *M. phaeos* as it did during conspecific contests (Tukeys HSD df=36, p=0.535; Tukeys HSD df=36, p=0.999), there was a significant reduction in aggressive acts toward heteromorphic *M. benetos* (Tukeys HSD df=36, p=0.001; Tukeys HSD df=36, p=0.004) and *M. callainos* (Tukeys HSD df=36, p=0.010; Tukeys HSD df=36, p=0.035).

Experiment 3

Metriaclima zebra performed half as many bites and lateral displays (Figure 4) toward the phylogenetically novel color pattern of *M.* 'msobo' than it did during territorial contests with conspecifics (ANOVA: $F_{1,18}$=7.952, P=0.011, ANOVA: $F_{1,18}$=6.735, P=0.018).

Discussion

While discussions of cichlid speciation typically focus on the importance of female choice (Turner & Burrows 1995, Higashi *et al.* 1999, Kondrashov & Kondrashov 1999, Takimoto *et al.* 2000, Danley & Kocher 2001, Lande *et al.* 2001), Kornfield and Smith (2000) recognized that intrasexual competition could be a powerful, yet largely unstudied force shaping the evolution of male color pattern within Lake Malawi. Seehausen and Schluter (2004) proposed that intense intrasexual selection directed towards homomorphic individuals could facilitate the invasion of a novel color pattern within a population, which could in turn lead to sympatric speciation. This study provides the first empirical support demonstrating that male aggression is preferentially directed towards individuals of a similar color pattern during territorial contests. Interactions between conspicuously heteromorphic males are less aggressive than those between homomorphic males. Further, territorial contests between homomorphic heterospecifics are as intense as conspecific interactions. The reaction of *Metriaclima zebra* toward a color pattern that is both geographically and phylogenetically unique suggests that the reduction in aggression observed during heteromophic contests is the inherent result of being presented with a novel stimulus rather than the product of an evolutionary process that occurs after secondary contact between heteromorphic species.

Field studies that focus on a generalized suite of behaviors associated with overall aggression (typically 'chases') find that most aggressive displays are directed toward heterospecifics (Holzberg 1978, Danley 2001). However, this result is not unexpected due to the diversity of targets for aggressive interactions in the field. These results are consistent with studies that focus on behaviors specifically associated with territory

39

acquisition (lateral displays, shivers, circling, tail beating). Holzberg (1978) observed that 'territorial fights' occurred only between individuals with a similar color pattern. Genner *et al.* (1999) found that male *Metriaclima* preferentially excluded conspecifics from their territories, but tolerated other congeners. Similarly, Hert (1990) reported that territorial *Metriaclima aurora* attacked conspecifics at twice the distance that they initiated attacks against heterospecifics. Differential aggression has even been used to delineate sympatric species of *Metriaclima* (Schröder 1980).

While competition for breeding territories is undoubtedly an important force shaping the evolution of male secondary characteristics, these results also suggest that intrasexual selection is not sufficient to explain the full range of color diversity observed among Lake Malawi cichlids. Allopatric variation in dorsal fin coloration has often been used to delineate species within the genus *Metriaclima* (Ribbink *et al.* 1983, Stauffer *et al.* 1997). However blue versus black dorsal fin coloration was not sufficient to reduce aggression during intraterritorial contests between *M. zebra* and *M. phaeos*. Similarly, Knight & Turner (1999) found that male *M. zebra*, *M. callainos*, and *M.* 'zebra gold' were able to differentiate conspecific females only when the female color patterns were conspicuously different (e.g. OB, white, brown). If female color patterns were similar, then males were unable to distinguish conspecifics from heterospecifics. Similarly, while female *M. zebra* and *M. benetos* can recognize conspecific males utilizing visual cues alone (Kidd et al. 2006a), female *M. emmilitos* were unable to distinguish between conspecific (BB, red dorsal) and *M.* 'Chilumba zebra' (BB, black dorsal) males unless both visual and chemical cues were available (Plenderleith et al. 2005).

These findings also suggest that in order for intrasexual selection to be the primary force driving differentiation, male color pattern must be controlled by a very small number of genes. Seehausen & Schluter's (2004) model and the results of this study suggest that male aggression is preferentially directed toward males with a similar color pattern. If the novel color pattern is polygenic, then the initial offspring produced from spawns between novel males and standard females will exhibit an intermediate color pattern. Since this intermediate morphology would likely include elements of both parental patterns, instead of experiencing a selective advantage during territory acquisition, these males might be recognized as conspecifics by both parental morphs. Even though intrasexual selection alone is not enough to explain the full range of color patterns observed among Lake Malawi haplochromines, it may be a powerful facilitating selective force when acting in conjunction with ecological or intersexual selection. In the presence of a novel 'open ended' female preference, the selective advantage received by males of a novel color pattern may help balance the intrasexual disadvantage incurred by intermediate males.

Studies of sexual selection in cichlid species often focus on the chromatic aspects of signals (Turner & Burrows 1995, Seehausen *et al*. 1997, Seehausen *et al*. 1998, Mann *et al*. 2004, Dijkstra *et al*. 2005). Indeed the importance of signal color during intrasexual interactions has been documented for a variety of cichlid species including *Cichlasoma meeki* (Evans & Norris 1996), *Astronotus ocellatus* (Beeching 1995), *Oreochromis niloticus* (Volpato *et al*. 2003), and *Amphilophus citrinellum* (Barlow 1973, McKay 1977, Barlow 1983). Conspecific mate choice between red and blue *Pundamilia* spp. can be disrupted under lighting conditions that mask male coloration (Seehausen *et al*. 1997, Seehausen & van Alphen 1998). Recently, Dijkstra et al. (2005) demonstrated that red *P*.

41

nyererei enjoy an advantage during intrasexual contests against blue *P. pundamilia*, which is eliminated when contests occur under monochromatic light. The results of this study suggest that melanistic pattern is more important than hue during intrasexual conspecific recognition between Lake Malawi cichlids. Similarly, Jordan et al. (2003) found that female *Metriaclima zebra*, *M. benetos* and *M. barlowi* (gold body, blue fins) were able to recognize conspecifics under monochromatic light, suggesting that pattern in the absence of hue can elicit conspecific recognition.

This study adds to a growing list of examples of fish species where the loss of bars in the body is associated with an increase in species aggression. Blue *M. benetos* and *M. callainos* males exhibited more aggression than either BB *M. phaeos* or *M. zebra* males during both conspecific and heterospecific contests. These observations are consistent with in situ observations of these species. Danley (2001) found that *M. benetos* was 3-4 times as aggressive as sympatric *M. zebra* at Mazinzi Reef, while Schröder (1980) found that *M. callainos* was twice as aggressive as *M. zebra* from Nkhata Bay. Territories of *M. callainos* are generally twice the area of those occupied by *M. zebra* (Holzberg 1978). Gold Midas cichlids (*Amphilophus citrinellum*) typically out-compete the more common 'barred' form for territories (Barlow 1973, Barlow & Ballin 1975), even though gold individuals do not appear to be inherently more aggressive (Barlow 1983). Males of barred swordtail species (*Xiphophorus* spp.) are typically less aggressive then males of closely related barless species (Moretz & Morris 2003).

The vertical barred pattern among fish species, or even within the family Cichlidae, does not appear to serve a 'universal' signaling function. Moretz & Morris (2003) suggest that the vertical bar pattern in swordtails (*Xiphophorus* spp.) is used as a

threat display designed to avoid conflict escalation by honestly conveying the senders motivation, or intention to attack. Similarly, vertical bars are associated with the intention to mouth-wrestle and may facilitate the coordination of assessment behaviors during intrasexual contests between males of the South American cichlid *Nannacara anomala* (Hurd 1997). The number of vertical bars appears to be an honest indicator of size in some species of *Xiphophorus* (Zimmerer & Kallman 1988), but not in others (Moretz & Morris 2003). In contrast, the barred pattern is associated with stress or submission in *Astronotus ocellatus* (Beeching 1995), *Oreochromis mossambicus* (Lanzing 1973), and *Tilapia mariae* (Slovin & Rowland 1978). If the barred color pattern serves purely as a threat or submissive display for the species observed in this study, then I would have expected to see consistently reduced or increased aggression directed towards males displaying the barred pattern during both conspecific and heterospecific interactions.

Acknowledgements

Table 2.1

Collection site, sample size and male coloration of species tested.

Metriaclima species	Collection Site	Generation in Captivity	(n)	Territorial Male Coloration		
				Body	Dorsal Fin	Caudal Fin
zebra	Mazinzi Reef	wild caught	20	Blue Barred	Blue	Blue
zebra	Mazinzi Reef	2,3	47	Blue Barred	Blue	Blue
phaeos	Undu Point	1	34	Blue Barred	Black	Blue/Yellow
benetos	Mazinzi Reef	1, 2	40	Blue	Blue	Blue
callainos	Nankoma Is.	wild caught	32	Blue	Blue	Blue
'msobo'	Magunga	1	8	Blue Blotched	Black	Blue/Black

44

Table 2.2
Results of BB vs. Blue territorial contests. Mean (standard error) of bites (black) and lateral displays (grey) observed during BB and Blue aggressive interactions/trials. Asterisks indicate significant deviation (ANOVA, $F_{1,18}$) compared to conspecific interactions (Bold).
* = **p<0.05**
**= p<0.01

Metriaclima	zebra	phaeos	benetos	callainos
zebra vs.	**132.0 (5.74)**	103.2 (2.27)	66.0 (3.68)*	76.2 (3.75)*
	40.3 (2.10)	38.9 (1.78)	16.4 (0.65)**	20.8 (1.03)*
phaeos vs.	102.9 (3.74)	**117.0 (3.75)**	71.9 (3.03)*	71.5 (3.22)*
	31.8 (0.96)	**37.5 (2.18)**	18.5 (1.39)	17.1 (1.26)*
benetos vs.	79.6 (4.39)**	97.6 (2.16)*	**157.4 (5.70)**	183.1 (7.07)
	14.6 (0.74)**	25.0 (1.33)*	**53.8 (2.63)**	50.3 (1.59)
callainos vs.	105.6 (3.84)*	89.5 (5.56)**	186.5 (8.94)	**173.4 (5.44)**
	22.8 (0.80)**	21.4 (1.49)**	45.5 (1.94)	**47.6 (2.04)**

45

Figure 2.1

Test arena used to assay intrasexual aggression during territorial contests. Bold lines indicate removable opaque barriers between adjacent 37.8 l aquaria. While double pane glass prevented auditory, tactile and olfactory communication between subjects, males had full visual access to females prior to testing, and to each other during testing.

100 cm

52 cm

| Conspecific Female | Male | Male | Conspecific Female |
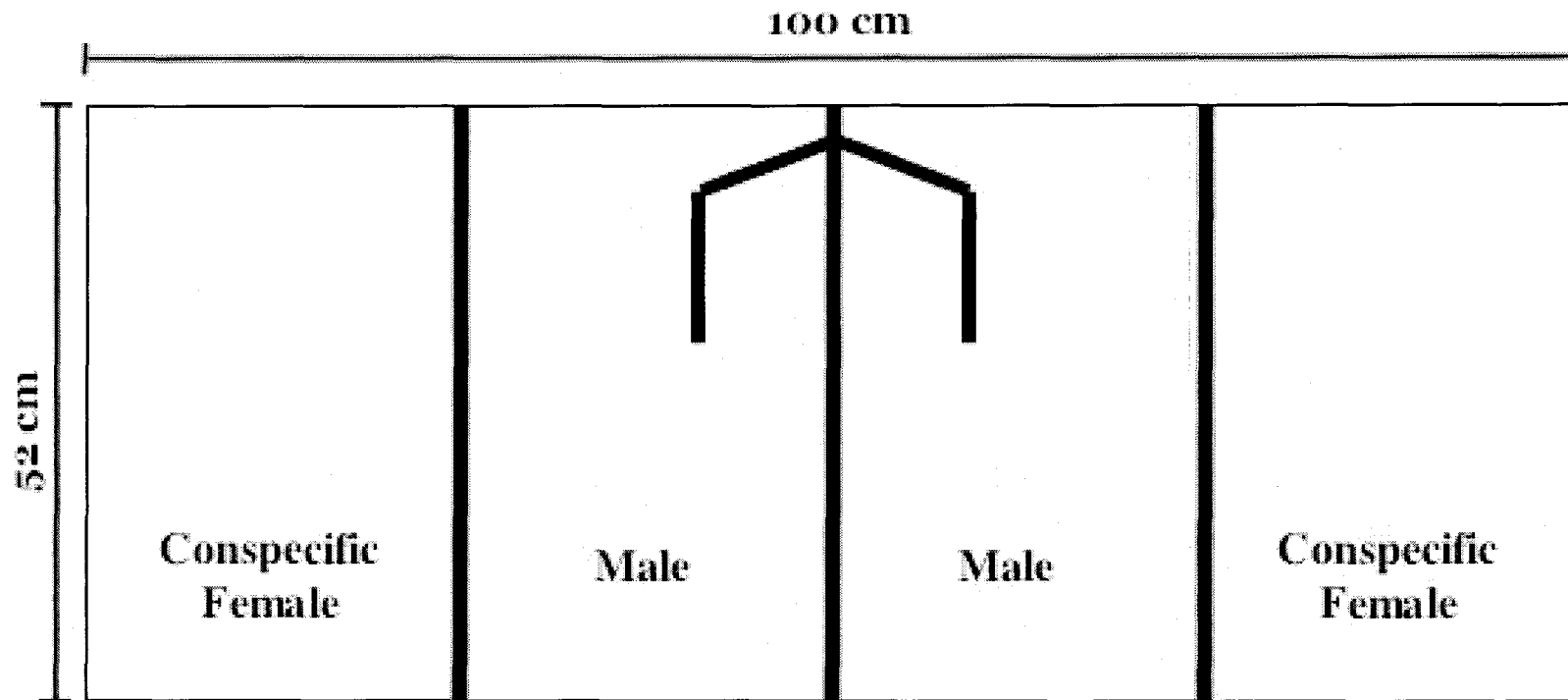
Figure 2.2

Box plot comparison of captive reared (2-3 generations) and wild caught *M. zebra* conspecific interactions. There is no significant difference between either number of bites (ANOVA: $F_{1,18}=1.067$, P=0.315) or number of lateral displays (ANOVA: $F_{1,18}=0.493$, P=0.491) performed by captive raised and wild caught subjects during intraterritorial contests. Box plots show the median (line), interquartile range (box) and minimum and maximum (whiskers) of the response values.
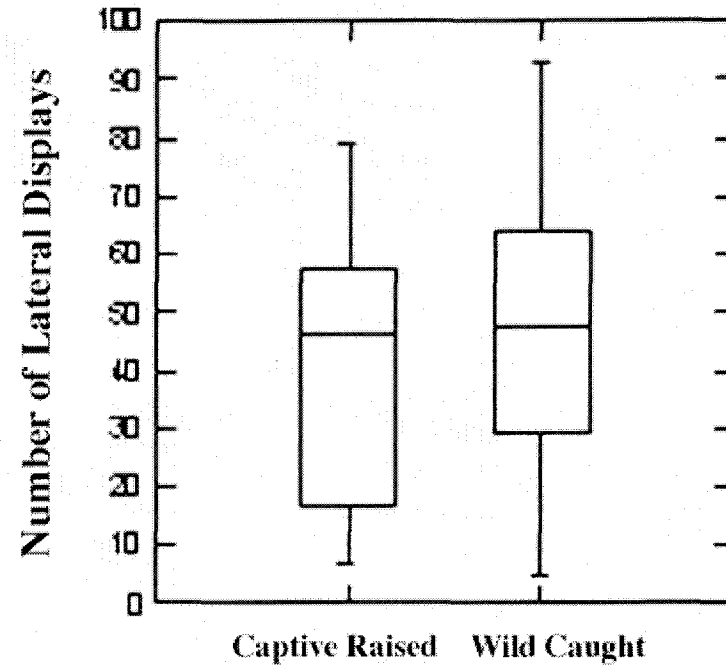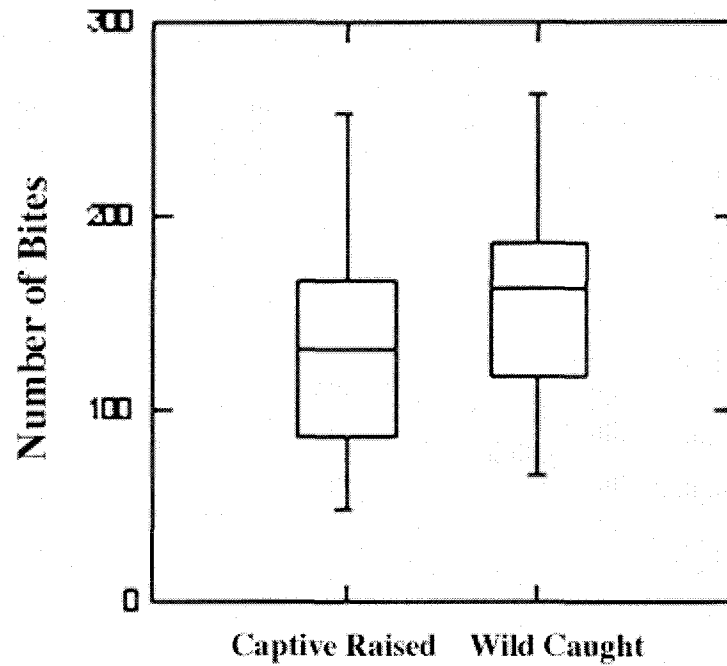
Figure 2.3

Box plot comparison of the number of bites and lateral displays during conspecific and heterospecific interactions between *Metriaclima benetos* (a), *M. callainos* (b), *M. phaeos* (c) and *M. zebra* (d). Red indicates the results of conspecific trials. The mean number of bites and lateral displays observed during territorial contests was significantly higher during homomorphic interactions versus hetermorphic interaction regardless of conspecificity (ANOVA, P<0.05 for all).
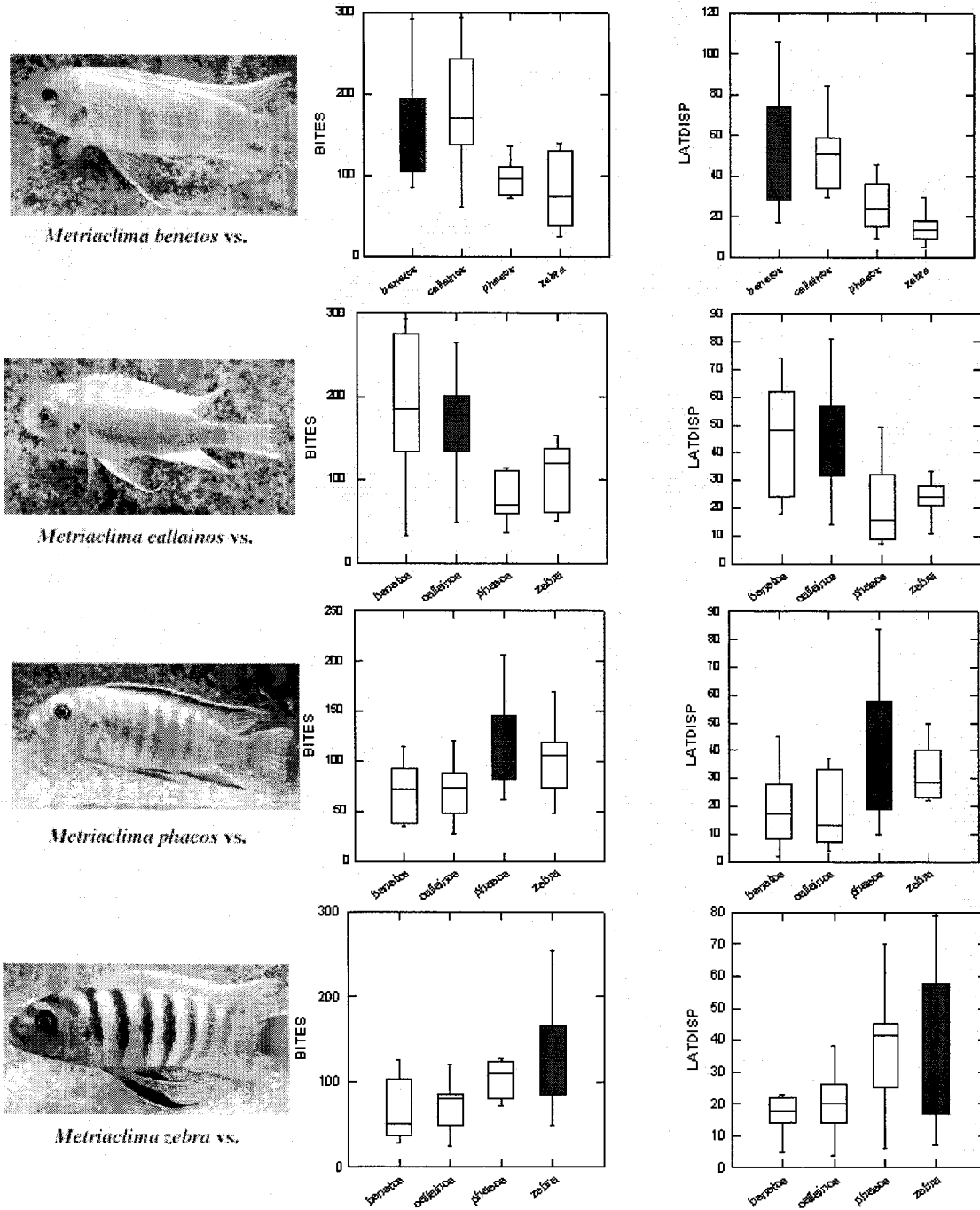
Figure 2.4

Box plot of the response of *M. zebra* to conspecific males and male *M.* 'msobo' Magunga. There is significantly less aggression directed at *M.* 'msobo' for both number of bites (ANOVA: $F_{1,18}$=7.952, P=0.011) and number of lateral displays (ANOVA: $F_{1,18}$=6.735, P=0.018).
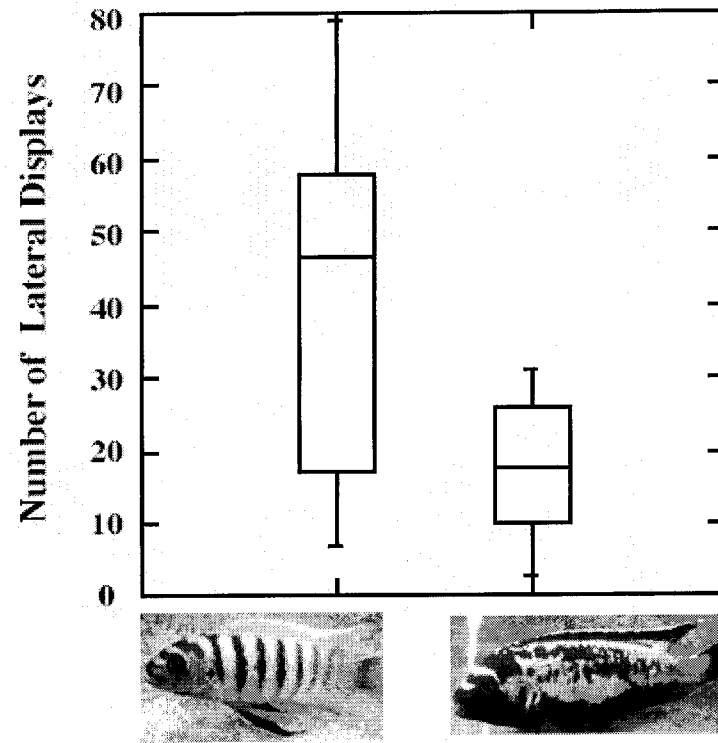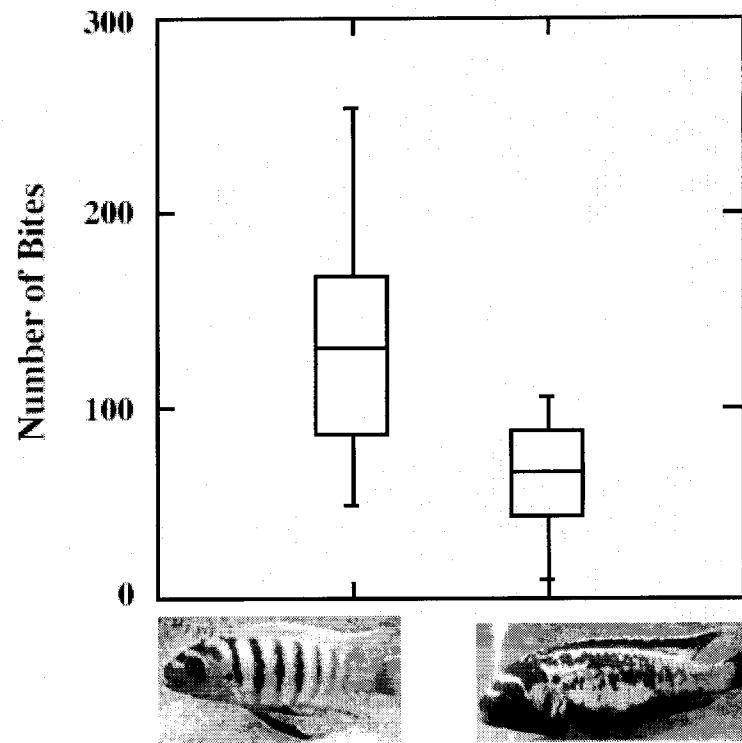
CHAPTER 3

SPATIAL AND TEMPORAL DYNAMICS OF A COMMUNITY OF TRANSLOCATED CICHLIDS AROUND THUMBI WEST ISLAND IN LAKE MALAWI

Abstract

The economic and ecological consequences of invasive species has prompted the

need to identify intrinsic and extrinsic factors that affect the dispersal of nonindigenous

species within a novel environment. During the 1960's and 70's, an ornamental fish

exporter captured numerous cichlid species endemic to northern Lake Malawi and

introduced them to the species rich rocky shoreline of Thumbi West Island within Lake

Malawi National Park. The translocation of 17 closely related species provides the

opportunity to evaluate the relative ability of species to disperse across a contiguous

habitat. A quarter of a century after their initial introduction, the translocated cichlid

community around Thumbi West Island is still in a state of extreme unrest. Many species

continue to spread around the island and have begun to colonize the mainland coast.

Rates of spread were found to differ both within and among species over time. The rate

of dispersal appears to depend on trophic utilization, population density, trophic

competition, and availability of suitable habitat. Specialized planktivores dispersed more

rapidly than generalist and algivorous species. The onset of species dispersal may be

triggered by intraspecific aggression for male spawning territories and is dependent on

50

population density, local species diversity, and the amount of suitable substrate. There is evidence that native *Labidochromis vellicans* is negatively impacted by competition with introduced algivorous *Labidochromis spp.*.

51

Introduction

Accidentally or with purpose, human activities have augmented the natural rate and scale of species dispersal. The transport, establishment and eventual spread of invasive species can cause irreversible changes to native communities with dire economic (Pimentel *et al.*, 2000) and ecological consequences (Lodge, 1993; Enserink, 1999; Parker *et al.*, 1999). Freshwater systems have proven to be particularly vulnerable to invasive organisms (Ricciardi & Rasmussen, 1998; Moyle, 1999; Rahel, 2000), which can drastically change ecological interactions (Townsend, 2003) and lead to the catastrophic loss of endemic fauna (Kaufman, 1992; Witte *et al.*, 1992). The staggering threat introduced species pose to global biodiversity has highlighted the need to identify both intrinsic and extrinsic factors which influence the ability of a species to become invasive (Moyle & Light, 1996; Ricciardi & Rasmussen, 1998; Higgins *et al.*, 1999; Kolar & Lodge, 2001; Rosecchi *et al.*, 2001; Marchetti *et al.*, 2004).

Elton (1958) suggested that the ability to become invasive depended on features of both the invading species and the invaded habitat. It is the interplay between abiotic and biotic factors that shapes assemblages of introduced and native species into discrete regional biotas (Vandewoestijne & Baguette, 2004). Abiotic factors that limit species dispersal (abiotic resistance) include the presence of geographical barriers, variability in suitable habitat, and distance from the founding population. Spatial distribution of an invading species is further shaped by biotic factors, which include inherent dispersal mechanisms (Perrins *et al.*, 1993; Ricciardi & Rasmussen, 1998), behavior (Holway &

52

Suarez, 1999), and predation (Baltz & Moyle 1993). Since many of these intrinsic features are likely to be variable between species, the rate of individual species dispersal is also likely to differ. Studies attempting to identify intrinsic characteristics that influence a dispersal ability often compare closely related species involved in "unintended experiments" (Rosecchi *et al.*, 2001; Marchetti *et al.*, 2004).

Lake Malawi's colorful rock dwelling cichlids (or mbuna) have become a textbook example of adaptive radiation (Kornfield & Smith, 2000; Kocher 2004; Genner & Turner, 2005). The incredible diversity of endemic color forms displayed by mbuna on the lake's rocky shores has drawn the attention of both evolutionary biologists and the aquarium trade. Between 1960-1975, the ornamental fish trade collected numerous mbuna species endemic to sites across Lake Malawi and transported them to a holding facility at Chembe Village. At least 17 species of cichlid endemic to Northern and Eastern Lake Malawi were released into the rocky habitat of nearby Thumbi West Island. While the exact date, relative timing, and population size of each introduction is unknown, all translocations occurred prior to the local cessation of export activities in 1975 (Ribbink *et al.*, 1983; Konings, 2000). High levels of species diversity and abundance at Mitande Rocks strongly suggest that this location was the site of introduction (Lewis, 1982; Ribbink *et al.*, 1983; Trendall, 1988). By 1980, the majority of species were still found in low abundance at Mitande Point and only two species had dispersed around the perimeter of the island (Ribbink *et al.*, 1983).

Unlike the disastrous introduction of Nile Perch into Lake Victoria (Kaufman, 1992), the impact of these translocated species on the native biota remains unclear. Some have suggested that these translocated species pose a threat to regional biodiversity

(Stauffer *et al.*, 1996; Barlow, 2000), however there has been no evidence of competition between Thumbi West's native and introduced inhabitants (Hert, 1995; Genner & Turner, 2005). Mitande Point's perpetually high levels of species richness is cited as evidence that the integration of these translocated species has occurred with minimal impact on the endemic fauna (Trendall, 1988; Turner, 1994; Genner & Turner, 2005). However, the documented hybridization of native *Metriaclima zebra* (Boulenger) with introduced *Cyanotilapia afra* (Günther) (Stauffer *et al.*, 1996) suggests that the translocation of species may have a profound impact on the genetic structure and evolution of endemic populations (Streelman *et al.*, 2004).

While deplorable, the intra-lacustrine transplantation of 17 closely related species provides a unique opportunity to evaluate the intrinsic and extrinsic factors driving species dispersal. Trendall (1988) observed that certain species of mbuna appear to have a superior ability to disperse. However, he was unable to separate this from the alternative hypothesis that the translocated cichlids spread at equal rates and that the observed distribution of species was a reflection of the extent of suitable habitat. Additional detailed surveys are required to separate these possibilities. Fifteen years after the last census, we performed a survey of the introduced species around Thumbi West Island in order to evaluate the perceived stability of this complex community and to identify biotic and abiotic factors that might have influenced the dispersal of these species. Finally, we looked for evidence of competition between native and introduced species of *Labidochromis*.

54

Methods and Materials

Thumbi West Island is located 1.5km northwest of Chembe Village in the southwest arm of Lake Malawi (Figure 1). The island's coastline consists of continuous rocky habitat interspersed with areas of sand and weeds (Trendall, 1988). The rocky habitat continues below the surface until it intersects with the underlying sand/mud substrate, which is often marked by sudden change in species composition. At Thumbi West Island the depth of this rock/sand interface ranges from 2-40m (Ribbink *et al.*, 1983) depending on location. Despite the recent addition of a small ecotourism station near Mitande Point, Thumbi West Island remains heavily forested, preserving clear water conditions that facilitate underwater observations. Prior to the island's incorporation into Lake Malawi National Park, the ornamental fish trade introduced approximately 17 species to the island's rocky habitat (Table 1).

Twenty-six line transects were established along the rocky coastline of Thumbi West Island (Figure 1). All transects were perpendicular to the coastline and positioned to approximate the location of previous surveys (Ribbink *et al.*, 1983; Trendall, 1988). Each transect was 33.5m long and 16 transects extended to the rock/sand interface ($\overline{X}\pm$SE=5.1$\pm$ 0.36m of depth). No transect areas overlapped. In order to minimize temporal fluctuation in distribution, all data were collected within a 2-week period in July 2001. Divers used SCUBA to perform two initial surveys to ensure consensus of species identification and to evaluate the suitability of specific locations. Since it is difficult to unequivocally identify *Melanochromis joanjohnsonae* (Johnson) from a distance, it was not surveyed during this study. In addition *Pseudotropheus socolofi* (Johnson), *P.*

55

livinstoni 'likoma,' *Metriaclima barlowi* (McKaye & Stauffer)(formerly *P. zebra*

'fusco'), and *Labeotropheus fuelleborni* 'yellow flank' were excluded from this survey

because no specimens were encountered during the initial reconnaissance.

Transects were censused by five observers that were each responsible for a

specific subset of 12 introduced (Table 1) and 1 native species (*Labidochromis vellicans*).

Divers descended in the same order and recorded the same species at each transect.

Territorial males of each species were recorded if they were observed within 1m on either

side of the transect line. Because they share a similar color pattern, both male and female

Metriaclima callainos (Stauffer & Hert) were recorded. Observers descended along the

transect line 2m above the substrate and at 10m intervals in order to reduce behavioral

disturbances. Several species were recorded by multiple divers in order to evaluate

interobserver reliability and to test the impact of observation on survey results.

Additional informal surveys were conducted at Mumbo Island, Otter Point and Domwe

Island, in order to document the spread of translocated species to adjacent rocky habitats.

In order to facilitate comparison of species abundance between surveys conducted

in 1980 (Trendall 1988) and 2001, species density was estimated using the King

estimator ($D=N/(2LR)$, where D is the estimated population density, N is the number of

individuals observed at each site, L is the length of the transect, and R is the detection

radius (Wright *et al.* 1999). Wilcoxon Signed Rank test was used to evaluate temporal

changes in species distribution around the island. Previous abundance data is not

available for *Cyanotilapia afra*, *Pseudotropheus tropheops* 'red cheek,' or any species of

Labidochromis, because Trendall (1983) did not survey these species and Ribbink *et al.*

(1983) only recorded the presence and absence of each species. A map of Thumbi West

56

Island (Ribbink *et al.*, 1983) was imported into NIH Image 1.59 to measure the linear distance between transects that were used to calculate species dispersal rates. If a species was present at two adjoining transects, then the species range was assumed to be continuous through the intervening area. If a species was observed at one transect and not the next, then species range was assumed to extend halfway between the two sites unless an obvious habitat discontinuity was present (e.g. 75m sandy beach between transects 14 and 15). Due to discontinuous dispersal along the northern coastline, all calculations were based on spread along the southern coast. Rate calculations also assume that all species were simultaneously introduced to Mitande Rocks in 1975, which likely inflates the rate of spread prior to 1980.

57

<u>Results</u>

Counts taken by multiple observers were highly correlated (Pearson, r range= 0.66 to 0.94, $\overline{X}\pm SE=0.81\pm 0.04$, p<0.02 for each comparison) suggesting high interobserver consistency and minimal behavioral impact from the census procedure. However, total abundance of *Metriaclima callainos* was significantly higher for the first observer along each transect (ANOVA: $F_{2,63}=14.831$, P <0.0001). Both males and females were counted for this species. Since females do not defend territories and spend much of their time feeding in the water column, they may be more susceptible to observational intrusion. Abundance and density measurements for *M. callainos* are based on the data collected by the initial observer, while all others are based on the mean of multiple observations.

A quarter of a century after their initial introduction, the translocated species at Thumbi West Island remain in a high state of temporal and spatial flux. As found in previous surveys (Ribbink *et al.*, 1983; Trendall, 1988), Mitande Rocks possesses the greatest diversity of introduced species (Figure 2). The southern coast harbors a greater number of introduced species per transect ($\overline{X}\pm SE=7.8\pm 0.15$) than the northern coast ($\overline{X}\pm SE=5.4\pm 0.09$; ANOVA: $F_{1,24}=12.211$, P =0.002), even though there is no difference in total abundance of introduced fish between the two coasts ($\overline{X}\pm SE=41.9\pm 1.07$ northern coast, $\overline{X}\pm SE=40.3\pm 0.85$ southern coast; ANOVA: $F_{1,24}=0.741$, P =0.74). Individual species of translocated mbuna continue to exhibit incredible variation in their current distribution and abundance around the island (Figures 3-5). A comparison with previous

58

surveys demonstrates that species have undergone both range expansion and contraction (Figures 6a-l).

Several species rapidly expanded their ranges to occupy the entire circumference of the island. *Metriaclima callainos* remained the most abundant introduced species at Thumbi West Island (Figure 3c). Since 1980, there has been no change in the overall range of *M. callainos* around the island (Figure 6k), however the distribution of species abundance has changed since 1985 (Wilcoxon Signed Rank, Z=-4.457, p<0.0001). Trendall (1988) found *M. callainos* to be more abundant along the southern coast, while we found no difference in abundance between the two coasts ($\overline{X}$±SE=15.6±0.34 northern coast, $\overline{X}$±SE=11.8±0.67 southern coast; ANOVA: $F_{1,22}$=1.948, P =0.177). *Metriaclima callainos* was also observed at nearby Otter Island in 2001 and 2005 and at Domwe Island in 2005.

Metriaclima aurora (Burgess) is found in a patchy distribution around the perimeter of the island (Figure 3b). Like *M. callainos*, there has been a change in the abundance of this species (Wilcoxon Signed Rank, Z=-4.457, p<0.0001), without a dramatic change in species range (Figure 6j). In 1985 the population density of *M. aurora* was centered around the Mitande peninsula and a bay on the northwest side of the island (Trendall, 1988). While we found the same increased population density at the northwestern bay (transects 23/24), we also found another center of abundance on the eastern shore (transects 15/16). This species frequents the intermediate habitat at the rock/sand interface (Hert, 1990), which predominates these sites (Ribbink *et al.*, 1983). *Metriaclima aurora* was also found in great abundance around Otter Point and Otter Island. While the spread of this species has been reported along the coast of the

59

Nankumba Peninsula to Tsano Rock (Stauffer & Hert, 1992; Konings, 2000), no individuals were observed at nearby Domwe Island in 2001.

Cyanotilapia afra has undergone the most dramatic increase of range observed during this survey (Figure 6a) and is currently found at all but two sites around Thumbi West Island (Figure 3a). Pinpointing when this dispersal began is difficult. This species was still limited to Mitande Point during Ribbink et al's (1983) survey in 1980. The survey performed in 1985 excluded species that were still limited to Mitande Point, but never specifically mentions C. afra (Trendall, 1988). Hert (1995) reports finding C. afra along the northern coast of the island (near transect17/18) in 1992, which is also when the first C. afra/Metriaclima zebra hybrids are found at Mitande Point (Stauffer et al., 1996). Recently Streelman et al. (2004) found that while the southern population of C. afra has hybridized with M. zebra, the northern populations have hybridized to a lesser degree. Cyanotilapia afra was also observed in abundance at Otter Island.

Two other species have recently circumnavigated Thumbi West Island. After a small expansion along the eastern shore, Pseudotropheus tropheops 'red cheek' was found in high abundance around the entire island (Figure 3d). This species has also been reported at Tsano Rock (Ribbink et al., 1983), but the origin of this population remains unclear. Surveys from 1980 and 1985 found Melanochromis parallelus (Burgess & Axelrod) to have patchy distributions around Thumbi West Island (Ribbink et al., 1983; Trendall, 1988). Trendall (1988) reported that the abundance of M. parallelus was centered at Mitande Point and a bay in the northwest corner of the island. While this survey found a similar concentration of population abundance in the northeast (transect 25/26, Figure 4b), M. parallelus has expanded its range (Figure 6h) along both the

60

southwest and northeast coasts of the island (Wilcoxon Signed Rank, Z=-3.832, p=0.0001) and has spread to Otter Point.

While they have not yet circumnavigated Thumbi West Island, several species did exhibit expanded ranges. *Melanochromis perileucos* (Bowers & Stauffer)(formerly *M.* 'black & white johanni') has continued its westward spread along both the north and southern shorelines (Figure 6i). Since 1985, the center of population density has shifted 500m westward along the southern coast (Figure 4c, Wilcoxon Signed Rank, Z=-3.219, p=0.0013). Individuals of this species were also common at Otter Point and Otter Island. While found in low abundance, both *Labidochromis* 'blue bar' (Figure 5a) and *L. strigatus* (Lewis, Figure 5e) have managed to spread westward along the southern coast (Figure 6h & 6f respectively). The most abundant representative of its genus (Figure 5c), *Labidochromis gigas* (Lewis) has dramatically expanded its range along the southern coast (Figure 6d). While not recorded along the transect line, *L. gigas* was also spotted on the northern coast of the island and at Otter Point.

Even as some translocated species appear to exhibit continued range expansion, other species appear to have suffered a reduction in range after their initial expansion. Despite an early expansion along the southern coast (Ribbink *et al.*, 1983), *Melanochromis interruptus* (Johnson)(formerly M. 'red') has experienced a gradual reduction in contiguous distribution (Figure 6g) and is currently restricted to Mitande Point (Figure 4a). Even though its contiguous range and abundance has been reduced, a few specimens have been observed previously at the far northwest corner of the island (Trendall, 1988) and at Otter Point during this survey. *Labidochromis pallidus* (Lewis) has had the greatest reduction of range of any species surveyed (Figure 6e). Originally

restricted to Mitande Point (Lewis, 1982), Ribbink *et al.* (1983) found this species in every transect. Ribbink *et al.* (1983) even suggested that this wide distribution and high abundance implied that *L. pallidus* was native to Thumbi West Island. However the current survey observed only a single specimen of *L. pallidus* at Mitande Point (Figure 5d). Also found in low abundance (Figure 5b), *Labidochromis freibergi* (Johnson) appears to be confined to Mitande Point. While this is a reduction from its earlier distribution along the southern coast (Ribbink *et al.*, 1983), a single specimen observed off-transect might suggest future expansion along the northern coastline (Figure 6c).

The only native species surveyed also exhibited a reduction in range (Figure 7). Found throughout southern Lake Malawi, *L. vellicans* (Trewavas) is one of the widest ranging and most abundant of the herbivorous *Labidochromis* (Lewis, 1982). Ribbink *et al.* (1983) found this species in every transect surveyed around Thumbi West Island. During this census, *L. vellicans* was found in only low abundance along the southern coast, but was more common along the northern coast ($\overline{X}\pm SE=0.2\pm0.04$ northern coast, $\overline{X}\pm SE=1.8\pm0.12$ southern coast; ANOVA: $F_{1,24}=15.621$, P $=0.0006$).

Rates of dispersal appear to vary between species (Figure 8). By 1980, while most species were still limited to Mitande Rocks, *Labidochromis pallidus, Metriaclima aurora, M. callainos*, and *Pseudotropheus tropheops* 'red cheek' had explosively dispersed along the southern coast (Ribbink *et al.*, 1983). While the ambiguity surrounding the exact date of each introduction may have overinflated these rates, *Melanochromis parallelus* and *Cyanotilapia afra* have both demonstrated similarly rapid rates of expansion within the documented period. In contrast, *Labidochromis* 'blue bar, *L. strigatus, L gigas* have experienced a slow but steady increase in range along the

62

southern coast. Planktivorous species dispersed more quickly when compared to algivores. In 1980, planktivorous and algivorous species have the similar mean ranges (ANOVA: $F_{1,10}=0.002$, P =0.964), however by 1985 (ANOVA: $F_{1,10}=9.238$, P =0.012) and 2001 (ANOVA: $F_{1,10}=8.552$, P =0.015) plantivorous species had double the mean range (Figure 9). Additionally, species range along the southern coast of Thumbi West Island was strongly correlated to population density (Spearman Rank, r=0.925, p=0.002).

Discussion

A quarter of a century of high species diversity at Mitande Point is often cited as evidence of the community's stability (Trendall, 1988; Turner, 1994; Genner & Turner, 2005). However replicated surveys over an extended temporal scale reveal that this community of translocated species exists in a continued state of flux. Within the past 20 years *Cyanotilapia afra*, *Melanochromis perileucos*, *Labidochromis* 'blue bar' and *L. gigas* have continued to spread around Thumbi West Island. *Melanochromis interruptus* and *M. parallelus* have both traversed 1.5km of open water and are currently found at Otter Point. After an extraordinary initial dissemination (Ribbink *et al.*, 1983), *Labidochromis pallidus* appears to have undergone an equally impressive collapse. Even species that have maintained consistent ranges over time demonstrate significant changes in the distribution of abundance within that range.

While Trendall (1988) suspected that species differed in their ability to disperse, he was unable to reject the alternate hypothesis that the observed distribution of species was simply the result of equal migration across a variable distribution of suitable habitat. The continued spread of translocated species since Trendall's survey strongly suggests that the extent of suitable habitat had not been reached in 1985 and that species differ in their innate ability to disperse. Our data indicates variable rates of dispersal, both between and within species over time (Figure 9). Dispersal rate appears to be dependent on a number of intrinsic and environmental factors.

It is commonly suggested that opportunistic feeders make good invaders (Lodge, 1993; Moyle & Light, 1996; Ricciardi & Rasmussen, 1998), however our results suggest

64

that this view is overly simplistic. While the omnivorous *Melanochromis parallelus* has successfully spread around the island, several species of trophic specialists display similar or greater range expansion. With the exception of *Ps. tropheops* 'red cheek', every species that has circumnavigated the island utilizes plankton as a major component of their diet (Ribbink *et al.*, 1983; Reinthal, 1990; Munthali & Ribbink, 1998). No introduced planktivore has failed to disperse around the island's perimeter. Moyle & Light (1993) suggested that omnivores/detritivores make good invasive species because they utilize a resource that is rarely limiting in aquatic ecosystems. Since primary productivity is higher in southern Lake Malawi (Eccles, 1974), plankton may not be a limiting resource for these species. However, Munthali and Ribbink (1998) found that while *Metriaclima callainos* at Thumbi West Island had higher fecundity and condition indices than at it's native location, the equally planktivorous *Cynotilapia afra* did not. Since species of mbuna are known to differ in their ability to compensate for rapid changes in depth (Hill & Ribbink, 1978; Marsh & Ribbink, 1981) it is likely that behavioral and physiological adaptations necessary to effectively feed in the water column may predispose certain species to increased dispersal. Marchetti *et al.* (2004) suggested that physiological tolerances were the best predictors of invading ability for fish. Recent studies have found that deep open water is a greater impediment to genetic migration between populations of algivorous *Labeotropheus fuelleborni* (Ahl) and *Melanochromis auratus* (Boulenger) than it is to planktivorous *Metriaclima zebra* (Danley *et al.*, 2000; Markert *et al.*, 2001).

Sakai *et al.* (2001) suggest that lag time between the initial introduction and the onset of rapid range expansion is the result of the time required for adaptive evolution to

overcome the genetic constraints of the colonizing species. Our results suggest the lag time for mbuna dispersal across a contiguous habitat is dependent on population density, local species diversity, and the amount of suitable substrate. Molecular data indicates that dispersal along contiguous rocky habitat is male biased (Knight *et al.*, 1999). Male mbuna fiercely defend territories from which they court passing females. Mbuna leks are often a mosaic of species shaped by competition between both conspecific and heterospecific males (Hert, 1995). Removal experiments have demonstrated that excised territorial males are usually replaced by conspecifics (Hert, 1990; Hert, 1995). This suggests that while intraspecific competition is high, interspecific competition for breeding territories is low. Excessive interspecific aggression is often cited as a key attribute of invasive species (Dick *et al.*, 1995; Gamradt *et al.*, 1997; Holway & Suarez, 1999). In contrast, the strong correlation between population density and range supports Trendall's (1988) suggestion that cichlid dispersal may be triggered by intraspecific competition for breeding sites once the local population density reaches some critical level.

The current distribution of *Labidochromis* species around Thumbi West Island may be shaped by competition for trophic resources. Modeling suggests that communities with high levels of resource partitioning will show greater resistance to invasion (Case, 1991). However Balz & Moyle (1993) found that competition was not an organizing factor for invasive fish species within the Sacramento-San Joaquin drainage of central California. While some have suggested that competition with translocated species may result in the extinction of Thumbi West's endemic fauna (Stauffer & Hert, 1992), Genner & Turner (2005) observe that if displacement is occurring, then it has been a slow

66

process. Our survey found that while *L. vellicans* has suffered a reduction of range in the south, both *L. gigas* and *L. strigatus* have made significant expansions, which supports Konings (2001) contention that the spread of introduced algivorous *Labidochromis spp.* has been impeded by competition with the ubiquitous *L. vellicans*. However, informal surveys in 2005 found *L. vellicans* in abundance in the shallows (1-2m) near transects 11 and 12. As the biotic structure of this community continues to change, additional behavioral and ecological data will be needed to evaluate the role of trophic competition in the distribution of these species.

Biotic factors alone do not entirely explain the distribution of species around Thumbi West Island. It is difficult to ascertain a biogeographical pattern of spread for many species due to the incredible rate of their initial dispersal. The biogeography of species exhibiting limited dispersal suggests that expansion is facilitated by the availability of suitable substrate. All of the translocated species have spread along the island's southern coast (Transects 3-13) which is a contiguous stretch of habitat dominated by an even cobble substrate (Trendall, 1988). Species that have expanded their range along the northern coast (*Labidochromis freibergi*, *L. gigas*, *Melanochromis parallelus*, *M. perileucos*, and *P. tropheops* 'red cheek') appear to have colonized the cobble habitat near transects17/18 prior to becoming established in the intervening area. The large sediment covered slabs and 100m expanse of sand along the eastern coast between transects 14-17 (Trendall, 1988) are likely a strong barrier to dispersal along the northern coast. This pattern would also be consistent with anecdotal reports of a second translocation event to the north. Since no unique species are observed along the northern

coast, the putative second translocation event must have occurred prior to Ribbink *et al.*'s (1983) survey and consisted of species already introduced to Mitande Rocks.

While it is difficult to identify a biological reason for the dramatic expansion and collapse of *Labidochromis pallidus*, there is ample evidence that such rapid fluctuations in population structure can occur within the period observed. Its initial range expansion is similar to those of the translocated planktivorous species, although *L. pallidus* is insectivorous. Phenotypic and genetic divergence of northern and southern populations of *C. afra* has occurred over a similar temporal scale (Streelman *et al.*, 2004). Historically, Lake Malawi has experienced severe climactic fluctuations (Eccles, 1974; Owen *et al.*, 1990), which may induce short-term environmental phenomena that change the relative abundance of trophic resources and increase competition between species. We have observed the potential for competitive exclusion driving the reduction of the native *L. vellicans*, however no obvious competitor to *L. pallidus* has undergone a compensatory increase in abundance. Long-term ecological research will be required to explain the dramatic temporal and spatial instability experienced by this species.

The simultaneous translocation of 17 cichlid species at Thumbi West Island presents a unique opportunity to study aspects of population biology within a well-studied evolutionary framework. Continued laboratory and field research will be required to tease apart the synergistic effects of intrinsic and extrinsic factors driving species dispersal. This will not only provide insights on the nature of invasive species, but also the forces shaping population structure within a model system for vertebrate speciation. While the potential for scientific exploration is exciting, our results also signal a cautionary tale. Despite the perceived stability of this system, our results indicate that

68

many of the translocated species have continued their spread unabated and threaten the continued existence of endemic fauna. The continued spread of translocated species along the mainland coast will further homogenize an ecosystem renowned for high levels of endemism (Ribbink *et al.*, 1983; Reinthal, 1993). While ornamental fish exporters no longer operate within Lake Malawi National Park, the recent availability of wild caught specimens native to these sites indicates that transient collections have resumed from within park boundaries. As the public demand for novel species drives fish exporters to move their operations along the northern and eastern shorelines of Lake Malawi, there is an increased risk to the celebrated biodiversity of this dynamic living laboratory of evolution.

Table 3.1

Species introduced to Thumbi West Island and source populations as reported by Ribbink *et al.* (1983) and Lewis (1982). An asterisk indicates that the species was also found at Otter Point or Otter Island by 1985. Diet composition data is from Ribbink *et al.* (1983) and Reinthal (1990).

Species	Source Location	Diet
Cynotilapia afra	Likoma Is.	zoo- and phytoplankton
Labeotropheus fuelleborni 'yellow flank'	Nakantenga Is.	NR
Labidochromis 'blue bar'	Namalenji Is.	various algae
Labidochromis freibergi	Likoma Is.	various algae, some plankton
*Labidochromis gigas**	Likoma or Chisumulu Is.	filamentous and other algae
*Labidochromis pallidus**	Maleri Islands	arthropods, filamentous algae
Labidochromis strigatus	Chisumulu Is.	arthropods, various algae
Melanochromis joanjohnsonae	Likoma Is.	NR
*Melanochromis perileucos**	Likoma Is.	plankton, benthic invertebrates
Melanochromis parallelus	Likoma or Chisumulu Is.	omnivore
Melanochromis interruptus	Chisumulu Is.	filamentous algae
*Metriaclima aurora**	Likoma Is.	plankton, various algae
Metriaclima barlowi	Maleri Islands	NR
Metriaclima callainos	Nkhata Bay	plankton, various algae
Metriaclima livingstoni 'likoma'	Likoma Is.	NR
*Pseudotropheus socolofi**	Mozambique coast	NR
Tropheops 'red cheek'*	Likoma Is.	filamentous algae, plankton

70

Table 3.2

Number of individual translocated species observed at each transect.

Transect	Cyanotilapia afra	"blue bar"	Labidochromis freibergi	gigas	pallidus	strigatus	vellicans	parallelus	Melanochromis perileucos	interruptus	Metriaclima callainos	aurora	Tropheops "red cheek"
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.00	0.00	0.00	24.00	0.33	3.50
2	0.33	0.00	0.00	1.00	0.00	0.00	0.00	3.00	0.00	0.00	14.00	1.00	5.50
3	0.00	0.00	0.00	2.00	0.00	2.00	2.00	4.00	3.00	0.00	23.00	4.00	10.50
4	2.00	0.00	0.00	1.00	0.00	0.00	0.00	2.00	1.00	0.00	2.00	3.33	5.50
5	5.00	1.00	0.00	4.00	0.00	0.00	1.00	2.00	5.00	0.00	21.00	1.67	4.00
6	1.33	0.00	0.00	4.00	0.00	0.00	0.00	3.00	4.00	0.00	16.00	4.33	7.50
7	2.67	0.00	0.00	1.00	0.00	0.00	0.00	0.00	5.00	0.00	14.00	1.33	5.00
8	4.33	3.00	0.00	2.00	0.00	2.00	0.00	0.00	16.00	0.00	17.00	0.33	5.50
9	4.33	1.00	0.00	1.00	0.00	0.00	0.00	0.00	16.00	0.00	23.00	1.67	2.50
10	5.67	1.00	0.00	3.00	0.00	2.00	0.00	0.00	10.00	0.00	26.00	1.00	6.50
11	2.33	2.00	0.00	0.00	0.00	1.00	0.00	1.00	5.00	0.00	4.00	2.33	7.50
12	3.00	2.00	1.00	0.00	0.00	2.00	0.00	1.00	6.00	1.00	10.00	1.67	6.00
13	11.00	1.00	3.00	9.00	0.00	1.00	0.00	2.00	0.00	1.00	13.00	0.67	3.00
14	8.67	3.00	2.00	4.00	0.00	1.00	0.00	0.00	0.00	0.00	15.00	0.33	5.50
15	5.00	0.00	0.00	0.00	0.00	0.00	3.00	1.00	0.00	0.00	8.00	9.00	4.00
16	3.00	0.00	0.00	0.00	0.00	0.00	2.00	4.00	0.00	0.00	13.00	5.50	3.00
17	4.50	0.00	0.00	0.00	0.00	0.00	2.00	9.00	0.00	0.00	36.00	0.00	4.50
18	2.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	30.00	0.00	7.50
19	3.00	0.00	0.00	0.00	0.00	0.00	3.00	4.00	0.00	0.00	40.00	0.00	4.00
20	3.00	0.00	0.00	0.00	0.00	0.00	1.00	6.00	3.00	0.00	38.00	0.00	4.00
21	5.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	2.00	0.00	17.00	0.00	2.50
22	6.50	0.00	0.00	0.00	0.00	0.00	3.00	0.00	3.00	0.00	11.00	3.00	7.00
23	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	9.00	0.50
24	2.00	0.00	0.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00	7.00	6.00	0.00
25	0.50	0.00	0.00	0.00	0.00	0.00	3.00	11.00	0.00	0.00	36.00	0.00	4.00
26	2.50	0.00	0.00	0.00	0.00	0.00	1.00	10.00	1.00	0.00	30.00	0.00	3.00

71

Figure 3.1

Transect position and approximate orientation along the northern and southern coasts of Thumbi West Island in southern Lake Malawi. Transects generally follow the previous surveys performed by Ribbink *et al.* (1983) and Trendall (1988).

72
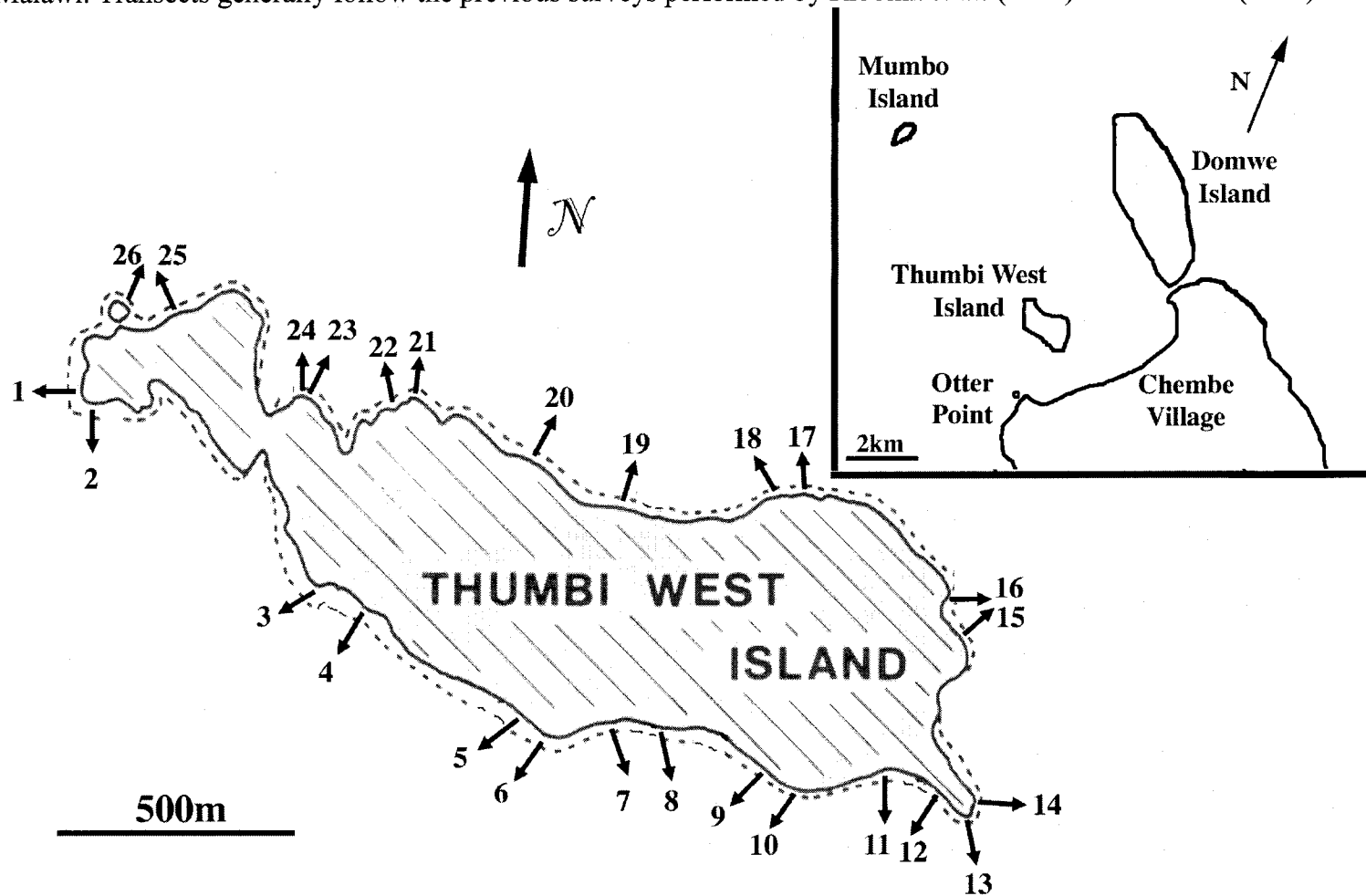
Figure 3.2
Distribution of introduced taxa around Thumbi West Island. Mitande Point (transects 13/14) continues to harbor the most diverse community of introduced species and 11 of 12 introduced taxa are present at these locations. While there has been an increase in the diversity of introduced species found at sites along the northern coast, transects along the southern coast still display greater overall diversity (ANOVA, p=0.002).
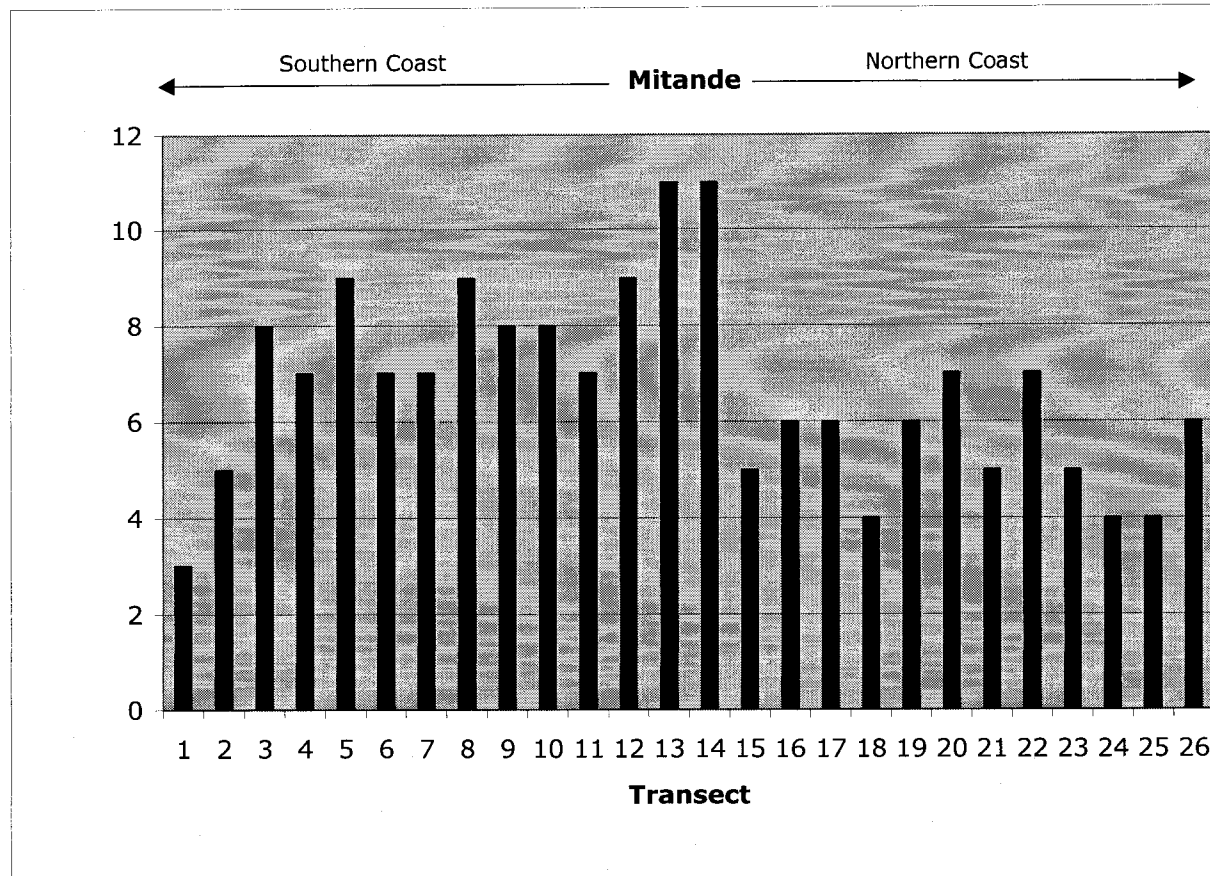
Figure 3.3

Current distribution and abundance of introduced *Cynotilapia afra* (a), *Metriaclima aurora* (b), *M. callainos* (c), and *Pseudotropheus tropheops* 'red cheek' (d) along the northern and southern coasts of Thumbi West Island. Transect locations are indicated by the circles and the presumed site of introduction (Mitande Point, transect 13) is represented by a double circle.

74
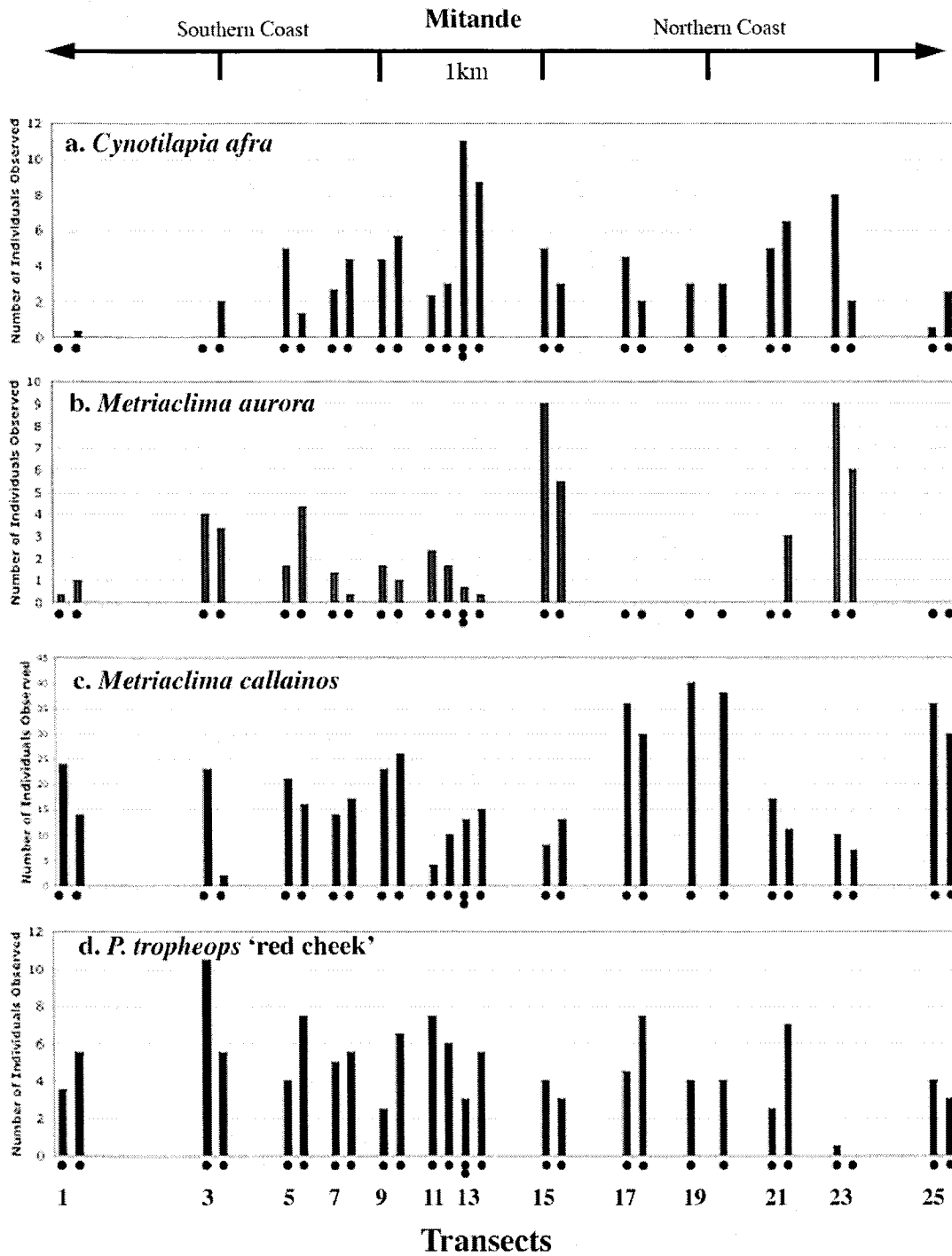
Figure 3.4

Current distribution and abundance of introduced *Melanochromis interruptus* (a), *M. parallelus* (b), and *M. perileucos* (c) along the northern and southern coasts of Thumbi West Island. Transect locations are indicated by the circles and the presumed site of introduction (Mitande Point, transect 13) is represented by a double circle.
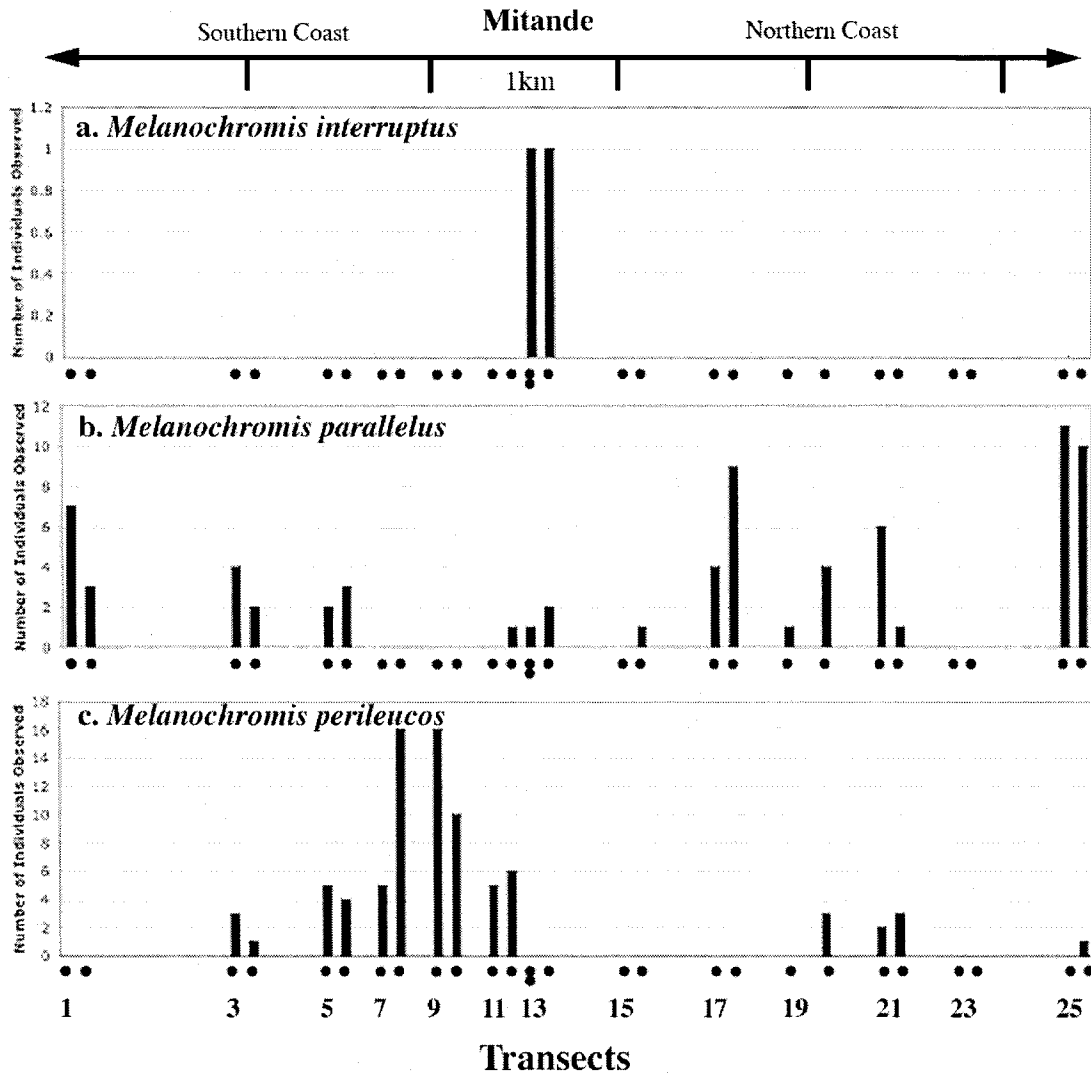
Figure 3.5

Current distribution and abundance of introduced *Labidochromis* 'blue bar' (a), *L. freibergi* (b), *L. gigas* (c), *L. pallidus* (d), and *L. strigatus* (e) along the northern and southern coasts of Thumbi West Island. Transect locations are indicated by the circles and the presumed site of introduction (Mitande Point, transect 13) is represented by a double circle.
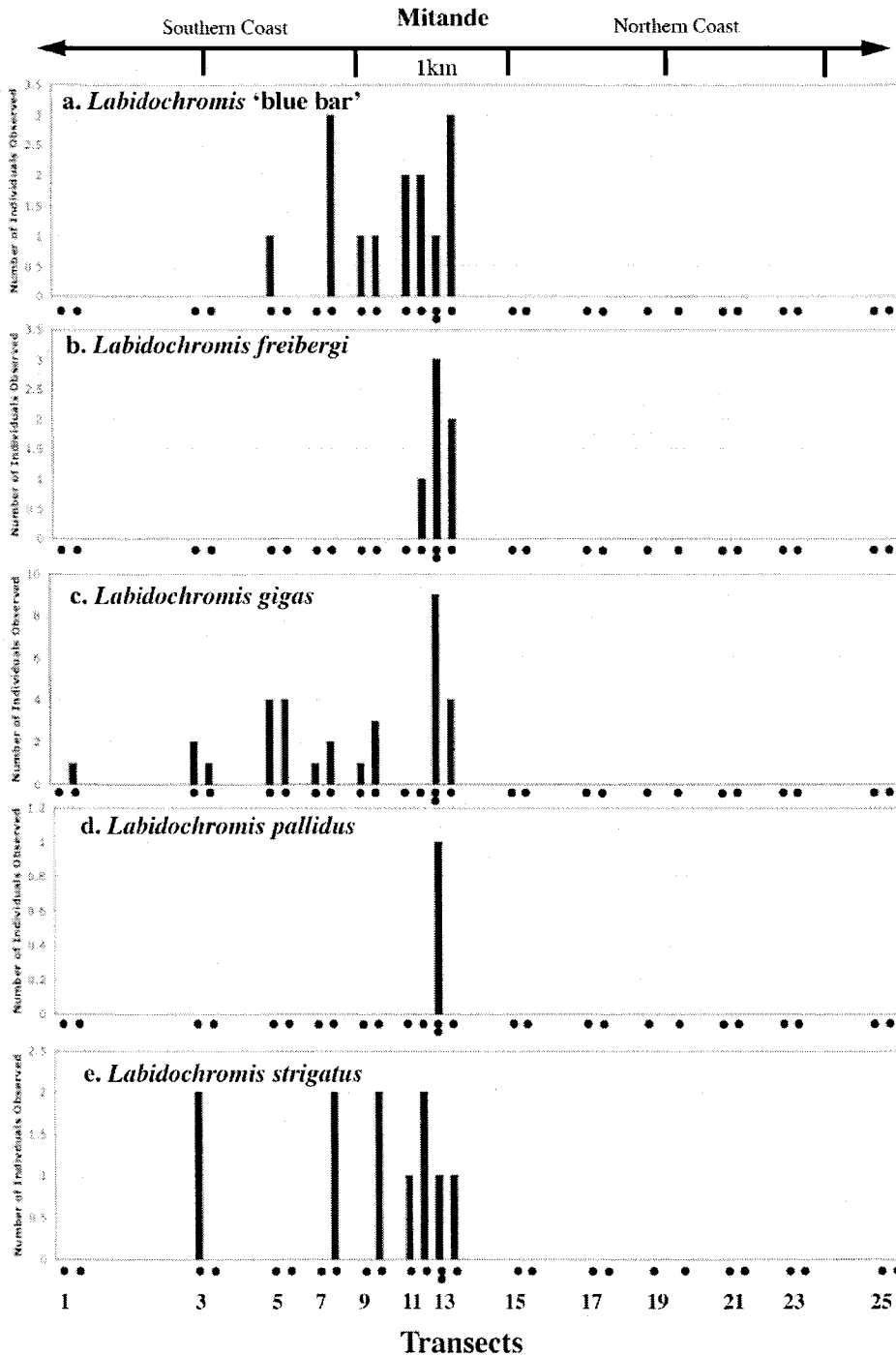
Figure 3.6

Spatial and temporal dispersal of each translocated cichlid species as surveyed at the time of introduction (red, hypothetical), in 1980 (yellow; based on Ribbink *et al.*, 1983), 1983 (green; based on Trendall, 1988), and 2001 (blue).

77
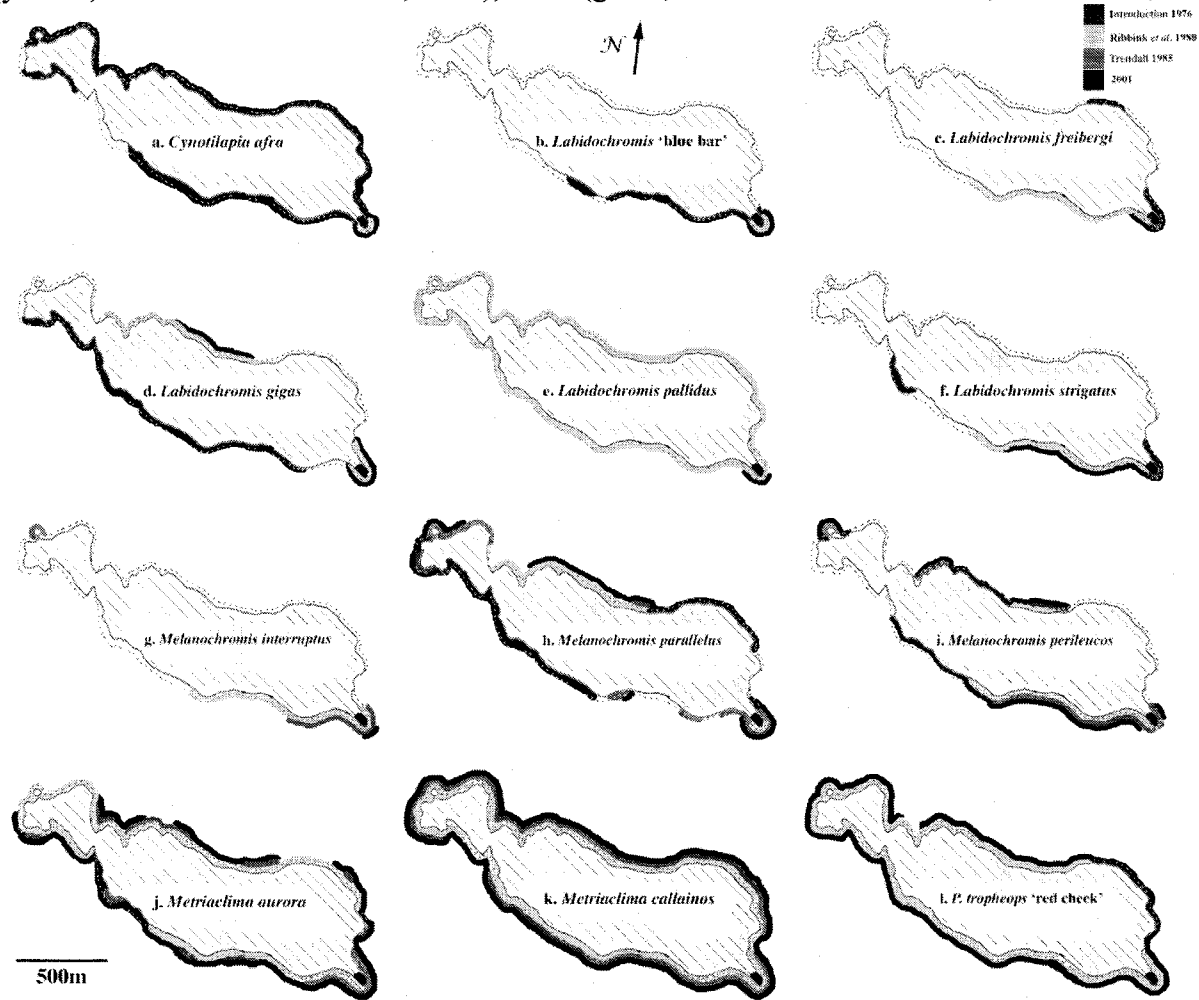
Figure 3.7

Current (a) and historical distribution (b) of native *Labidochromis vellicans*. Transect locations are indicated by the circles and the presumed site of introduction (Mitande Point, transect 13) is represented by a double circle. Historical distribution of *L. vellicans* as surveyed in 1980 (yellow; based on Ribbink *et al.*, 1983) and 2001 (blue).
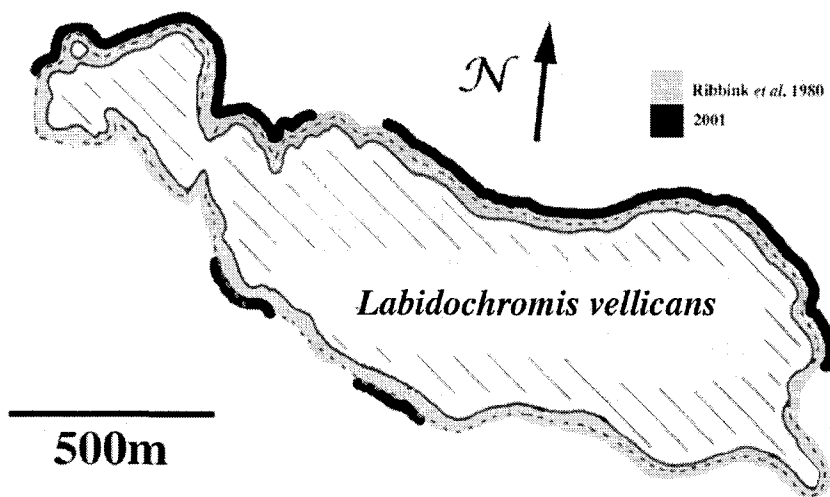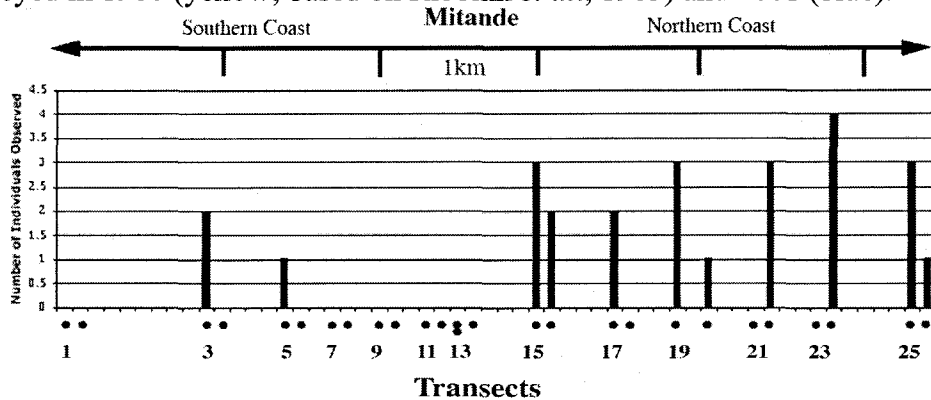
Figure 3.8

Expansion of species range along the southern coast of Thumbi West Island since the cessation of export activity in 1975. Several species show continued expansion, while others show no movement and even a reduction from earlier ranges.
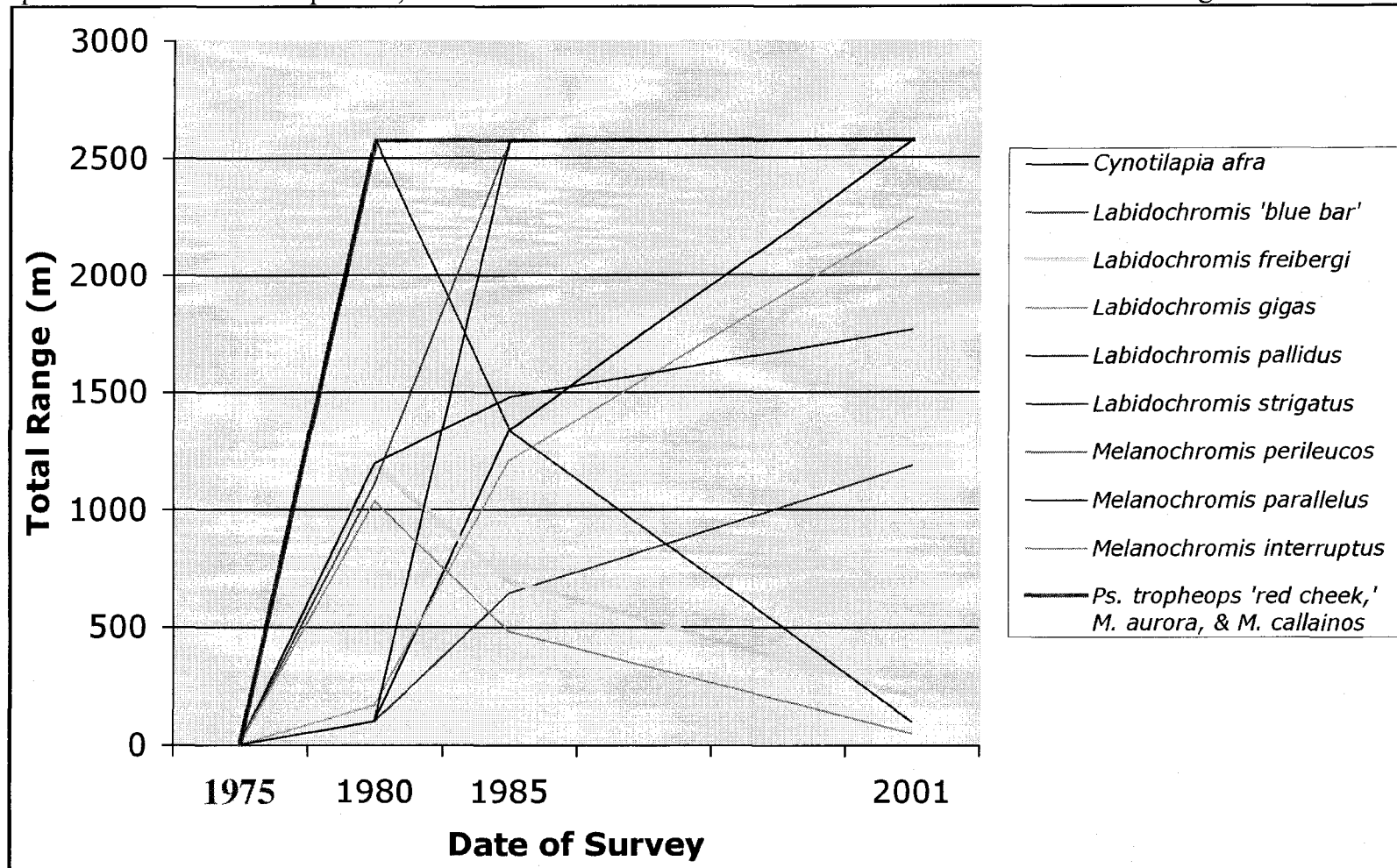
Figure 3.9

Comparison of mean range between plankivorous and algivorous translocated species. Both trophic guilds had similar mean ranges in 1980 (ANOVA, p=0.964), however by 1985 and into 2001 plantivorous species have double the mean range held by algivorous species (ANOVA, p<0.05 for both 1985 and 2001).

Comparison of Mean Species Range between Trophic Guilds
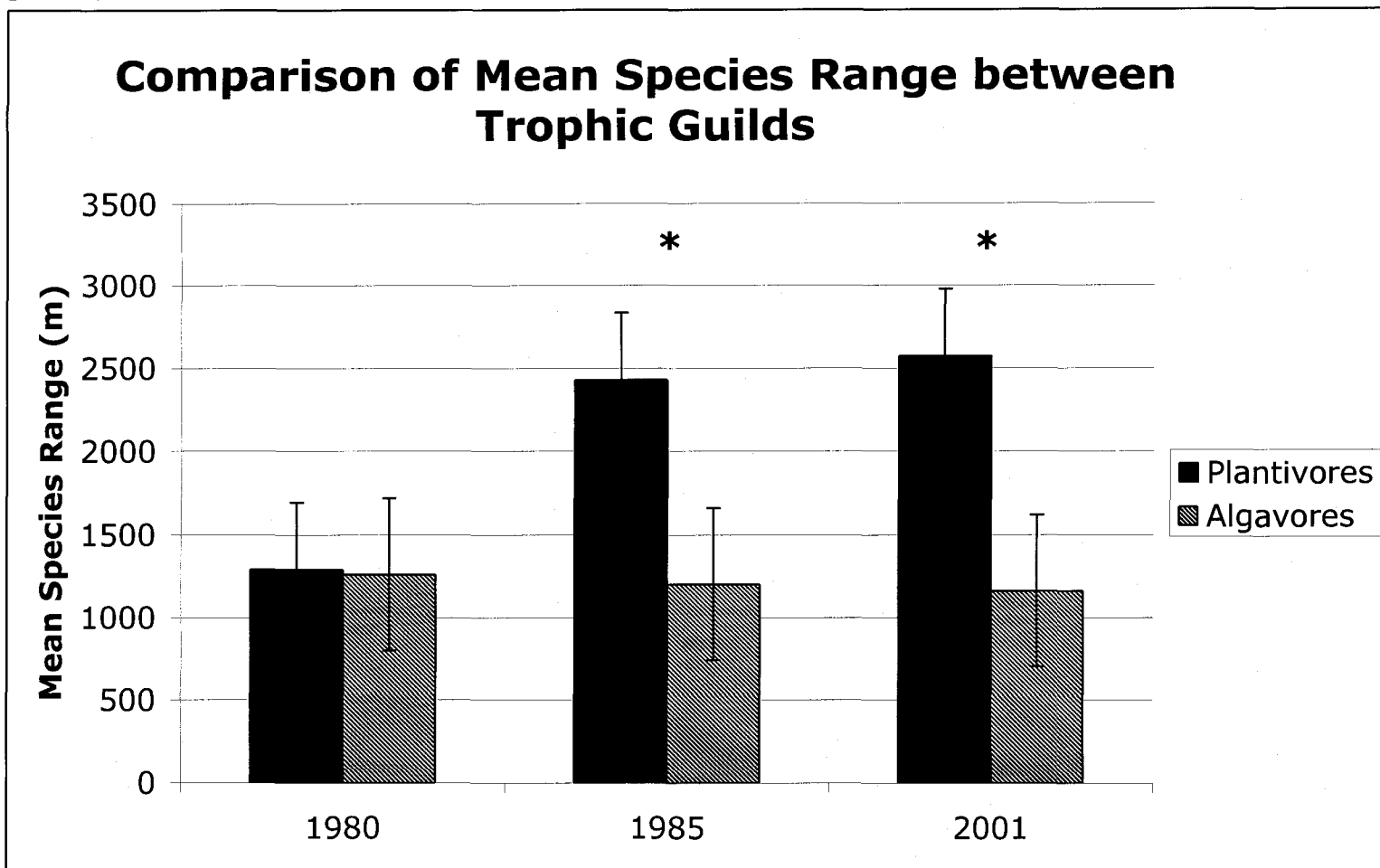
CHAPTER 4

PATTERNS OF DIVERSIFICATION WITHIN *PSEUDOTROPHEUS TROPHEOPS*, A SUBGENUS OF ROCK DWELLING CICHLID FROM LAKE MALAWI.

Abstract

The haplochromine cichlids of Africa's Great Lakes have become a celebrated example of adaptive radiation. Unfortunately, phylogenetic analysis of the Lake Malawi species flock has been confounded by the lack of appropriate morphological characters and an exceptional rate of speciation, which has allowed ancestral molecular polymorphisms to persist within species. To overcome this problem I used Amplified Fragment Length Polymorphism (AFLP) to score more than 7,000 restriction fragment polymorphisms in order to reconstruct the evolutionary relationships among 20 species of the ubiquitous rock dwelling subgenus *Pseudotropheus tropheops* along the southeast arm of Lake Malawi. The results suggest that this lineage forms a monophyletic clade that should be elevated to full generic status. I found that cladogenesis is associated with numerous unambiguous changes in male color states, reiterating the importance of color differentiation during recent speciation events. A geometric morphometric analysis of the lower jaw of these taxa reveals the repeated differentiation of jaw morphology associated with species habitat preference. The parallel and rapid evolution of trophic morphology, habitat preference and male color pattern suggest that while sexual selection is a

81

ubiquitous force acting during recent cladogenic events, natural selection continues to act both independently and synergistically during the diversification of the *Pseudotropheus tropheops* complex. By investigating the relative role of natural and sexual selection during recent cladogenic events I evaluated the predictions of three competing models of cichlid speciation.

Introduction

In the absence of direct observation and experimentation, unraveling the forces acting during speciation is often challenging. However phylogenetic analysis of species rich lineages provides useful insights into the forces that operate during population divergence and the formation of reproductive isolation (Barraclough *et al*. 1998, Losos & Glor 2003). In both magnitude and speed, the parallel intralacustrine radiations of East African cichlids are a premier example of explosive speciation and have emerged as a powerful model of vertebrate evolution (Kornfield & Smith 2000, Kocher 2004, Genner & Turner 2005). Within Lake Malawi an estimated 1,000 species have evolved from a single riverine ancestor within the past 1 million years (Meyer *et al*. 1990). The dynamic cladogenesis of Lake Malawi's endemic fauna has coincided with an astounding diversification of trophic morphology, feeding behavior and male color pattern, implying that multiple evolutionary forces have been important during the evolution of this lineage.

This remarkable lineage represents both an opportunity and a challenge to evolutionary biologists. It has inspired numerous models that attempt to identify the forces acting during cichlid speciation. Analysis of microsatellite variation indicates that rates of gene migration between fragmented rocky habitats may be sufficient to preclude genetic drift as a principle mechanism for population divergence (Danley *et al*. 2000, Markert *et al*. 2001). Therefore most models focus on the action of natural and/or sexual selection during speciation. While several models suggest that speciation can occur by sexual selection alone (Lande 1981, Turner and Burrows 1995, Wu 1985, Higashi *et al*. 1999, Takimoto *et al*. 2000, Lande *et al*. 2001), some authors contend that speciation

without niche divergence will lead to extinction by competitive exclusion (van Doorn *et al*. 1998, Arnegard & Kondrashov 2004). Since models invoking only a single evolutionary force would fail to explain the full range of trophic morphology and color diversity observed among the rock dwelling cichlids (mbuna) of Lake Malawi, many competing models postulate the synergistic actions of multiple forces during speciation (eg. Mayr 1984, Kondrashov & Kondrashov 1999, Danley & Kocher 2001). Since each of these models differ in the temporal action of natural and sexual selection, each also differs in the expected delimitation of characters related to ecology and mate choice in extant pairs of sister taxa.

Dominey's (1984) hypothesis of non-adaptive speciation and the reproductive allopatry exhibited by other lacustrine fish species (Smith and Todd 1984), led Mayr (1984) to propose that reproductive isolation is rapidly generated between allopatric populations of cichlids via divergent female choice on male sexual characteristics. Mayr's (1984) Smith-Todd-Dominey model (STDM) suggests that when gene flow is limited between adjacent allopatric populations, Fisherian sexual selection generates divergent mate recognition systems. Subsequently when behaviorally isolated "mating clusters" come into secondary contact, ecological differentiation is driven by competition over shared resources. The STDM would predict that recently diverged taxa within a lineage would differ primarily by sexually selected traits. Since ecological divergence occurs secondarily, some taxa would exhibit differentiation and others would not. Mayr (1984) further suggested that the apparent defiance of Lake Malawi cichlids to the rules of competitive exclusion was the result of very recent and rapid speciation. He further

observed that the cichlids of Lake Tanganyika, which are among the oldest lineages of east African cichlids, also exhibit the greatest trophic diversity.

In contrast, the Neutral Marker Model (NMM) proposes that color diversity may not be the result of sexual selection, but instead acts as an independent markers that facilitate assortative mating of ecotypes during trophic divergence. If divergence occurs in sympatry, then speciation requires the evolution of prezygotic mechanisms for reproductive isolation (Tregenza and Butlin 1999). Assortative mating can be based on the ecologically selected trait, or a neutral marker phenotype (Dieckmann and Doebeli 1999, Kondrashov and Kondrashov 1999). Dieckmann and Doebeli (1999) suggest that marker phenotypes, which are neutral to natural selection, can be co-opted as prezygotic reproductive barriers. They further suggest that even if the loci controlling the ecological and marker phenotypes are not initially linked, selection against ecological hybrid deficiency will increase the association of independent marker loci with genes controlling trophic morphology. Since assortative mating based on color pattern facilitates and occurs concurrently with trophic diversification, this model predicts that all recently diverged sister taxa should be differentiated for both ecological and color characteristics.

Danley and Kocher's (2001) Three Stage Model (TSM) suggests that natural and sexual selection have acted sequentially during three primary periods of cladogenesis. Initial divergence occurred when a generalist riverine ancestor colonized different intralacustrine habitats (pelagic, rock, sand). A subsequent radiation, initiated by ecological competition within these habitats, produced the currently recognized genera of trophically distinct mbuna. Most recently, sexual selection by female choice has resulted in the parallel diversification of male color pattern within each trophic lineage. While this

85

model has received some criticism (Genner & Turner 2005), it has also been applied to Lake Tanganyika's parallel cichlid radiation (Salzburger & Meyer 2004) as well as other species rich vertebrate lineages (Streelman & Danley 2003). Danley and Kocher's TSM predicts that recently evolved sister taxa within a lineage will be divergent in sexual selected characters (male color pattern) and should show limited or no ecological differentiation.

Testing the predictions of these models requires robust phylogenetic hypotheses of recently evolved taxa. High levels of intraspecific variation (Lewis 1982) and rampant convergent evolution (Kocher *et al.* 1993, Reinthal & Meyer 1997) has confounded the construction of cichlid phylogenies via traditional cladistic analysis of morphological characters and has impeded our understanding of the forces acting during speciation (Mayr 1984, Stiassny 1991, Kornfield & Smith 2000). Whether due to the retention of ancestral polymorphisms (Moran & Kornfield 1993, Kornfield & Parker 1997) or the result of rampant hybridization (Seehausen *et al.* 2004, Schliewen & Klee 2004), data generated from a single or small number of loci have been unable to resolve species level phylogenies of Lake Malawi cichlids (Kornfield & Parker 1997, Kocher 2003). Techniques that survey thousands of independent nuclear loci, such as Amplified Fragment Length Polymorphisms (AFLP), have emerged as the primary tool for elucidating the relationships between recently evolved cichlid species (Albertson *et al.* 1999, Allender *et al.* 2003, Seehausen *et al.* 2003, Schliewen & Klee 2004, Kassam *et al.* 2005, Kidd *et al.* 2006b).

In order to evaluate the predictions of three competing models of cichlid speciation (STDM, NMM, TSM) I use AFLP markers to reconstruct the evolutionary

86

history of a ubiquitous lineage of rock dwelling cichlid along the southeast arm of Lake Malawi. Species within the mbuna subgenus *Pseudotropheus tropheops* share a rigid subterminal mouth used to "nip" algae from rocks while hovering above the substrate at a 45 degree angle (Ribbink *et al*. 1983). These species are among the most abundant cichlids observed at any rocky habitat (Fryer 1959, Ribbink *et al*. 1983) and have the widest distribution of any mbuna around Lake Malawi (Reinthal 1993). Even though these species appear to occupy a similar trophic niche, *P. tropheops spp*. display a wide variety of habitat preferences (depth, amount of sediment, rock size), which coincide with specific differences in the shape of the lower oral jaw that may indicate microhabitat partitioning (Albertson 2002). Using landmark-based morphometrics (Albertson & Kocher 2001), I examined variation in jaw shape and habitat preference in order to assess ecological differentiation between pairs of recently evolved sister taxa. Within this lineage I also examined the complex evolution of male color patterns, which is critical to conspecific mate recognition (Knight *et al*. 1998, Couldridge & Alexander 2002, Jordan *et al*. 2003, Kidd *et al*. 2006a).

Materials and Methods

Sample collection and field work

Populations of the subgenus *Pseudotropheus tropheops* were sampled along the southeast arm of Lake Malawi, Africa during July and August 2001 (Figure 1, Table 1). Unlike the deeper main body of Lake Malawi, the southeast arm is much more shallow and has likely experienced a dynamic history of lake level fluctuations (Owen *et al.* 1990, Kornfield & Smith 2000). Using coalescent data, Won *et al.* (2005) recently estimated divergence times of 1,000 to 17,000 years for three species of *Pseudotropheus tropheops* within this region. Even though *Pseudotropheus tropheops* was one of the first mbuna to be described (Regan 1921), 44 of the 50 individual varieties currently recognized (Ribbink *et al.* 1983, Konings 2001) lack formal description. Therefore species identification and nomenclature follow Ribbink *et al.* (1983) and Konings (2001).

All samples were taken from live fish collected using SCUBA and monofilament nets, with the exception of *Tropheus duboisi*, which were purchased through the aquarium trade. Immediately upon capture, photographs of each specimen's left side were taken and color patterns were scored (black, white, orange, yellow, blue, grey, and brown) for discrete external regions (Figure 2b) following the terminology of Ribbink *et al.* (1983) and Barel *et al.* (1977). Fin clips were taken from the right pectoral fin of each specimen and stored in 95% EtOH. Three to five voucher specimens were preserved in 10% formalin and later transferred to 70% ethanol for long-term storage. Additional specimens collected for subsequent morphological analysis were dried on site.

Morphological analysis

Dried specimens for morphometric analysis were cleaned of tissue and disarticulated using a colony of dermestid beetles (Table 1). The lower oral jaws were bleached with 10% H_2O_2 and oral and pharyngeal jaw dentition was recorded. Digital photographs of the lateral lower oral jaws were taken with a SPOT digital camera (Diagnostic Instruments, Inc.) mounted on a Zeiss SV11 dissecting scope. The photographs were imported into NIH Image 1.59 and landmark positions were scored as Cartesian (x,y) coordinates (Figure 2a). Shape of the lower jaw was quantified using geometric morphometerics following Albertson & Kocher (2001) and Albertson (2002). The landmark coordinates were imported into MORPHOMETRIKA 7.0 (Walker 2000) and superimposed using a generalized least-squares fit algorithm to remove variation due to size, orientation and translation. Shape variables were obtained by generating partial warp scores using thin-plate spline analysis (Rolf 1993). The shape variables were reduced using a principle component analysis (PCA) on the partial warp scores in order to identify differences in jaw shape between species. Multivariate analysis of variance (MANOVA) was used to evaluate differences in jaw shape among species and were followed by Tukey's test to evaluate univariate pair-wise differences between species and replicate populations.

Molecular techniques

Genomic DNA was extracted from the fin clips of 3 individuals of each taxon (2 for *Melanochromis auratus*, *P. tropheops* 'lilac' from Thumbi West Island, and *Tropheus dubosi* from Lake Tanganyika) using a standard phenol/chloroform extraction (Wang *et*

89

al. 2003). AFLP is a DNA fingerprinting technique that characterizes thousands of restriction polymorphisms spread throughout the genome (Vos *et al.* 1995). In this method, genomic DNA is first double-digested using two restriction enzymes (in this case EcoRI and MseI). Double stranded adapters are then ligated onto the "sticky" ends of the fragments. An initial "preselective" PCR reaction amplifies a subset of fragments that match adapter primers containing an additional nucleotide (EcoRI-A and MseI-C). This product of this pre-selective amplification is then used as the template for 18 different "selective" amplifications performed with primers containing an additional 2-nucleotide extension (Table 2). The detailed protocols can be viewed at (http://hcgs.unh.edu/protocol/basic).

Fragments were separated using a Beckman Coulter CEQ 8000 capillary sequencer. Peaks were scored using a quartic model with a slope threshold of 2.0% and relative peak height of 5.0%. All samples of *P. tropheops* spp., *T. dubosi* and *Rhamphochromis* spp. were run concurrently, however samples of *Metriaclima* spp., *Cynotilapia afra* and *Melanochromis auratus* were run in a separate electrophoresis. Bands were scored as present/absent using Beckman Coulter's Fragment Analysis Module. The presence of each fragment was confirmed manually. Fragments between 80-500 bp in size were binned (1 nucleotide bin width) using Beckman Coulter's AFLP Analysis Software. The binary output was imported to an Excel spreadsheet and formatted for PAUP 4.0b8 (Swofford 2001).

90

Phylogenetic Analysis

Matrices were generated by calculating Nei and Li's (1979) genetic distance. The phylogram was constructed from 1,000 bootstrap replicates using a neighbor joining algorithm in PAUP v.4.0b8 (Swofford 2001). The data were tested for hierarchical structure by analyzing the frequency and distribution of tree lengths for 1,000,000 randomly generated trees (Hillis & Huelsenbeck 1992). Other studies have reported gene flow between species of the subgenus *Pseudotropheus tropheops* (Won *et al.* 2005), so we evaluated the effects of reticulation on the structure of this phylogeny using the homoplasy excess test (Seehausen 2004, Schliewen & Klee 2004) and SplitsTree v.2.4 (Hudson 1998) following Kidd *et al.* (2006b). Habitat and depth preference (from Ribbink *et al.* 1983) and all color characters were imported into MacClade v4.06 (Maddison & Maddison 2003). We examined the number of unambiguous changes per color character in order to identify specific areas of the body that exhibit high levels of transition. The parallel evolution of male coloration was tested by designing constraint trees that assumed a monophyletic origin for each color state, using the most variable characters (chin, shoulder, dorsalateral band, chest, midlateral band, caudal peduncle, dorsal fin, pelvic fin, and anal fin). Using the same parameters described above, PAUP v.4.0b8 identified the best tree that included the constraint. Shimodaira-Hasegawa tests (SH) were used to compare the alternate topological hypotheses (Shimodaira & Hasegawa 1999).

91

The Phylogeny

Eighteen primer pair combinations generated 7,953 characters ($\overline{X}$=441.8 per

primer pair, Table 2). Of these, 5,853 were polymorphic and informative ($\overline{X}$=328.0 per

primer pair). Bootstrap support increased with the number of characters (primer pairs)

added (R^2=0.0770, p>0.0001). A plot of the length of 1,000,000 random trees

demonstrated significant non-random structure to the data set (g_1= -0.8171, 87 samples,

p<0.01). These data were used to construct a distance tree (Figure 3). The entire tree has

a mean bootstrap value of 83.0% and 63 nodes were resolved above 75% (Figure 4).

Replicate samples from the same population cluster together with a mean bootstrap value

of 98.4%. All species form monophyletic clusters (supported by $\overline{X}$=98.2% bootstrap

support) with the exception of *Pseudotropheus tropheus gracilior* and *Pt.* 'intermediate,'

which appear to be periphyletic with respect to *Pt.* 'Otter gold' and *Pt.* 'Mumbo zebra'

respectively.

This tree recovers all of the expected relationships between outgroup taxa from

previous overlapping phylogenies. *Tropheus dubosi* a haplochromine from Lake

Tanganyika was used to root the tree. The genus *Rhamphochromis* emerges as basal to all

other Lake Malawi samples, which corresponds to previous phylogenies based on

mtDNA (Kocher et al. 1993, Meyer 1993) and AFLP (Allender *et al.* 2003, Kidd *et al.*

2006b) data. The mbuna species *Cynotilapia afra* and *Melanochromis auratus* are basal

to all taxa previously assigned to the genus *Pseudotropheus* (including *Metriaclima*).

This is in sharp contrast with the recent AFLP based phylogeny of Kassam *et al.* (2005),

which found that *Metriaclima* (*Maylandia*) *zebra* was sister to 7 taxa within the genus

Cynotilapia to the exclusion of *Pseudotropheus tropheops* 'orange chest and *Pt*. 'red cheek.' Unfortunately the samples of *C. afra* and *M. zebra* used by Kassam *et al.* (2005) were both collected from Thumbi West Island where these species have been hybridizing for at least 10 years (Stauffer *et al.* 1996, Streelman *et al.* 2004). The relative position of *C. afra*, *M. auratus* and all of the *Metriaclima* spp. in this phylogeny may have been confounded by their placement in a separate electrophoresis, however this topology does recover the relationships expected by traditional taxonomy, which until recently placed all *Metriaclima* and *P. tropheops* species within the same genus (Stauffer *et al.* 1997).

Several authors have suggested that the *Pseudotropheus tropheops* species complex represents a monophyletic lineage that should be given full generic status (Reinthal & Meyer 1997, Konings 2001, Genner & Turner 2005). In contrast, Fryer (1959) suggested that the characters used to delimit these species represent an unusually high level of intraspecific variation and synonymized all of the existing species under *Pseudotropheus tropheops*. This phylogeny clearly resolves the previously delimited taxa of *Pseudotropheus tropheops* irrespective of location, indicating that species at a given location are independently derived and reproductively isolated. Additionally the taxa of *Pseudotropheus tropheops* sampled along the southeast arm of Lake Malawi all form a strongly supported (bootstrap=100%) monophyletic lineage, lending support to their elevation to full generic status.

The number of observed instances of cichlid hybridization continues to increase (Stauffer *et al.* 1996, Seehausen *et al.* 1997, Salzburger *et al.* 2002, Smith *et al.* 2003, Schliewen & Klee 2004, Streelman *et al.* 2004, Kidd *et al.* 2006b). While Won *et al.* (2005) reported instances of hybridization between *P. tropheops* spp., both Seehausen's

(2004) homoplasy excess test and the split decomposition technique (Hudson 1998) failed to identify any instances of reticulation within this data set. Both of these methods are very sensitive to excess homoplasy generated by hybridization. Cassens *et al.* (2003) found that split decomposition revealed network topology for a data set that yielded unambiguous topology when analyzed with either statistical parsimony or median joining algorithms. Schliewen and Klee (2004) used the homoplasy excess test to identify reticulation undetected by Canonical Ordination. Additionally both of these tests identified matching instances of hybridization within an AFLP based phylogeny of sand dwelling cichlids from Lake Malawi (Kidd *et al.* 2006b). While hybridization may be a factor during the evolution of some cichlid lineages (Salzburger *et al.* 2002, Seehausen *et al.* 2003, Schliewen & Klee 2004), these results suggest that it has not been a significant factor during the diversification of these taxa.

Evolution of Male Color Patterns

The species surveyed in this study display a wide range of complex and evolutionarily labile color patterns (Figure 5). Of all of the color characters scored, only gular color, number of egg spots, dorsal and anal fin submarginal band, and dorsal and anal fin leading edge coloration appears to be conserved (2 or less changes). All other color characters have undergone 6-10 ($\overline{X}\pm SE = 6.43\pm0.157$) unambiguous changes during the diversification of this lineage. Additionally, very few of the color states within these rapidly evolving characters define a monophyletic lineage (Table 3), indicating the repeated parallel evolution and/or loss of specific male color patterns within this lineage.

94

Several authors have argued that the parallel evolution of color characters observed among species within the mbuna genus *Metriaclima* is a strong indication of the ubiquitous role of male color pattern during cichlid speciation (Smith & Kornfield 2002, Allender *et al.* 2003). The evolution of male color pattern during the divergence of the *Pseudotropheus tropheops* lineage has been even more dynamic and complex. This phylogeny clearly resolves sympatric taxa that were initially identified by divergent male color patterns, further supporting the importance of color pattern in delimiting specific taxonomic status (Lewis 1982, Ribbink *et al.* 1983, Stauffer *et al.* 1995, Stauffer *et al.* 1997, Turner 2000). Cladogenesis is associated with numerous unambiguous changes in male color states (ranging from 0-13 unambiguous changes, $\overline{X}\pm SE=3.25\pm0.189$, Figure 6).

The role of female mate choice on male coloration has become a central tenent of speciation models dealing with Lake Malawi cichlids (Dominey1984, Mayr 1984, McKaye 1991, Turner & Burrows 1995, Deutsch 1997, Seehausen *et al.* 1999, Danley & Kocher 2001, Lande *et al.* 2001, Turner 2001, Kocher 2004, Genner & Turner 2005). Even though it is common to find multiple congeners that differ in male color pattern at any given location (Ribbink *et al.* 1983, Koning 2001), few hybrids have been observed in the wild. The ease with which interspecific and intergeneric fertile hybrids are produced in captivity (Crapon de Caprona & Fritzsch 1984, Albertson *et al.* 2003) underscores the importance of pre-zygotic behavioral isolation toward maintaining reproductive isolation. Since courtship and spawning behaviors appear to be highly stereotyped and similar among mbuna species (McElroy & Kornfield 1990), isolation based on divergent courtship behaviors is unlikely. Therefore recent studies have focused

on the role of divergent female preference on male color pattern in initiating and maintaining reproductive isolation. The importance of male color pattern on female conspecific recognition is well documented (Knight *et al.* 1998, Couldridge & Alexander 2002, Kidd *et al.* 2006a). Female mbuna appear to be able to identify conspecific males when visual cues alone are available (Kidd *et al.* 2006a). Pauers *et al.* (2004) found that female *Labeotropheus fuelleborni* preferred males with increased color contrast and saturation. Additionally, female conspecific recognition is disrupted when color cues are masked by aberrant lighting conditions (Seehausen *et al.* 1997, Seehausen & van Alphen 1998). The patterns of divergence observed in this study are entirely consistent with the expectation of rampant and repeated divergence of male color pattern facilitated by sexual selection.

Most haplochromine cichlid species have a cluster of yellow/orange ovoid spots on the anal fin that are commonly referred to as egg spots or egg dummies. These spots are thought to facilitate the oral fertilization of eggs held in the mother's mouth (Wickler 1962). Several studies have demonstrated that the number of egg spots can affect male reproductive success. Hert (1986, 1991) found that female cichlids display a preference for males with more egg spots. In contrast, Couldridge (2002) found that female *Metriaclima lombardoi* demonstrate a preference for males with only a single egg spot. Female preference for increased number of egg spots and the natural variability of egg spot number among and within haplochromine species led Goldshmidt & de Visser (1990) to suggest that these color characters may act in conspecific recognition and further argued that the repeated divergence in egg spot number has driven the explosive speciation exhibited by these species. While the number of egg spots on the anal fin is

96

one of the few color characters previously identified to directly affect male reproductive success (Hert 1991), this trait appears to be highly conserved within this lineage. All of the species and populations surveyed during this study exhibited 2-3 egg spots on the anal fin, which corresponds with all previous color descriptions for these species (Regan 1921, Ribbink *et al.* 1983, Koning 2001). While intraspecific variation in egg spot number and preference may facilitate the initiation of reproductive isolation in some haplochromine lineages, it is clearly insufficient to explain the diversification of the *Pseudotropheus tropheops* species complex.

Evolution of Jaw Shape

Fryer (1959) suggests that cichlid assemblages represent a "peaceful condominium" because the superabundance of algae ameliorates the competition between species. However the variation is jaw shape observed in this study suggests that microhabitat partitioning continues to play a critical role in the structure of mbuna communities. A multivariate analysis of variance identified significant differences in jaw shape among species (MANOVA, Wilks' λ=0.2637, $F_{12,212}$=16.65, p<0.0001) along principle components (PC)1 and 2, which explained 39% and 20% of the variation respectively (Figure 7a). Pair-wise comparisons (Tukey HSD df=212) identified significant differences in jaw shape between several species along PC1 and PC2 (Table 4). Comparisons between replicated populations (Tukey HSD df=115) identified significant differentiation between the Otter Island and Thumbi West populations of *Pt.* 'intermediate' as well as between the Zimbawe Rock and Domwe Island populations of *Pt.* 'orange chest' (Table 5). No replicate populations of the same species were

97

significantly differentiated along PC2. The differentiation observed along PC1 is typical

of changes in mandibular morphology that result in higher (positive along PC1) versus

lower (negative along PC1) mechanical advantage (Albertson et al. 2005). These

morphological changes are associated with biting (high mechanical advantage) and

sucking (low mechanical advantage) modes of feeding in many percoid lineages

(Wainwright 1996, Westneat 1995, Alfaro *et al.* 2004). While variation in oral (Fryer &

Iles 1972, Poll 1986, Tichy & Seegers 1999, Streelman *et al.* 2003) and pharyngeal jaw

(Smits *et al.* 1996, Barluenga & Meyer 2004, Kidd *et al.* 2006b) dentition are typically

associated with ecological subspecialization among recently evolved cichlid taxa, oral

and pharyngeal jaw dentition was identical to previous descriptions for the subgenus *P.*

tropheops (Regan 1921, Fryer 1959, Ribbink *et al.* 1983) and uniform among all of the

populations surveyed along the southeast arm of Lake Malawi.

Several authors have observed that sympatric congeners of *Pseudotropheus*

tropheops differ in depth distribution and preference for sediment rich and sediment poor

habitats (Ribbink *et al.* 1983, Reinthal & Meyer 1997, Albertson 2002). Our results

indicate that variation in jaw shape along PC1 is strongly associated with species depth

distribution. Species that prefer shallow habitats (less than 10m) tend to have jaws with

higher mechanical advantage and are strongly differentiated along PC1 (ANOVA, $F_{1,220}$ =

191.023, p<0.00001) from species that prefer deeper habitats (greater than 10m) and

possess jaws with lower mechanical advantage (Figure 7b). Analyses of stomach contents

confirm that species inhabiting different depths also have different diets. Reinthal and

Meyer (1997) found that stomach contents of shallow species of *P. tropheops* contained

attached algae (*Cladophora, Calothrix*) while the stomachs of deeper dwelling species

contained diatoms and pelagic algae that settle on rocks. Neither preference for deep (SH p<0.0001) nor shallow habitats (SH p<0.0001) defines a monophyletic lineage indicating the parallel origin of this ecological trait (Figure 8).

Our results are equally consistent with differentiation of jaw shape between species that occupy sediment rich and sediment poor habitats (ANOVA, $F_{1,220} = 177.238$, p<0.00001). Sediment coverage of rocks tends to increase with depth (Ribbink *et al.* 1983, Albertson 2002). Albertson (2002) found that sediment coverage, rather then habitat depth, was a better predictor of ventral jaw shape among *P. tropheops* species. This study includes limited morphological samples (Table 1) of the 3 species that deviate from the deep/sediment rich-shallow/sediment poor rule (*Pt.* 'black dorsal', *Pt.* 'broadmouth', and *Pt.* 'yellow gular'). Additional sampling of these species will be required in order to separate the effects of habitat depth and sediment coverage on the evolution of *P. tropheops* jaw morphology.

Meyer (1987) suggested that phenotypic plasticity could explain the majority of morphological variation observed within the African species flocks. However, this was predicated on the assumption that New and Old World cichlids displayed a similar magnitude of plasticity. Meyer further noted that the results of Witte (1984) suggested that the effect of plasticity was less pronounced in Old World lineages, a result further corroborated by Smits *et al.* (1996) and Huysseune (1995). While Stauffer & Gray (2004) found that live versus commercial diets induced significant differences in the external head morphology of Lake Malawi species, they also found that the magnitude of plasticity was much less than that of New World cichlids reared on the same diets. Using the same morphological landmarks utilized in this study, Albertson et al. (2003)

demonstrated that several quantitative trait loci (QTL) correlated to the functional

divergence of jaw morphology between *Metriaclima zebra* (biting) and *Labeotropheus*

fuelleborni (sucking) evolved in response to strong directional selection. Regardless of

the mechanism of morphological differentiation, our data indicate that ecological forces

are exerting selective pressure on the jaw shape of closely related species.

Which Speciation Model Applies?

The patterns of divergence revealed by this phylogeny are most consistent with

Mayr's (1984) Smith-Todd-Dominey model (STDM) in which sexual selection initiates

behavioral reproductive isolation and is followed by ecological differentiation of closely

related taxa after secondary contact triggers competition for trophic resources. Since

trophic competition acts secondarily, this model predicts that while all recently diverged

taxa within a lineage would differ in male color pattern, only some would exhibit

ecological differentiation. This study found that divergence of sister taxa within the

Pseudotropheus tropheops species complex always coincides with multiple unambiguous

changes in color character states (Figure 6). The repeated parallel evolution of male color

pattern within this lineage reiterates the importance of divergent sexual selection during

recent cladogenic events (Smith & Kornfield 2002, Allender *et al.* 2003). Additionally,

while ecological differentiation of habitat preference and jaw shape has occurred during

the diversification of this lineage, it does not appear to be necessary for speciation. While

both *Pt.* 'Mumbo zebra' and *Pt.* 'lilac Mumbo' are ecologically differentiated from the

closely related *Pt.* 'intermediate,' the well supported pairs of sister taxa *Pt.* 'Otter

gold'/*Pt. gracilior*, *Pt.* 'broadmouth'/*Pt.* 'Black dorsal' and *Pt.* 'Chinyankwazi'/*Pt.*

'Chinyamwezi' exhibit no significant differences in jaw shape (Table 4). These results are in strong concordance with a recent phylogenetic analysis of Lake Malawi's sand dwelling lineage, which found that while every pair of sister taxa constructed different male display platforms (bowers), only half of the taxa also differed in oral or pharyngeal jaw dentition indicative of trophic subspecialization (Kidd *et al.* 2006b). While Mayr (1984) proposes that speciation commences with divergence of male color patterns between allopatric populations as the result of Fisherian 'runaway' sexual selection, these results are equally consistent with other proposed models of allopatric or sympatric speciation via sexual selection (Lande 1982, Turner & Burrows 1995, Wu 1985, Higashi *et al.* 1999, Takimoto *et al.* 2000, Lande *et al.* 2001, Kocher 2004, Seehausen & Schluter 2004) followed by secondary ecological differentiation.

While patterns of divergence revealed by this phylogeny are most consistent with Mayr's (1984) STDM, this model may not be sufficient to explain all of the patterns of differentiation revealed by this study. Speciation is possible without the divergence of jaw shape, however ecological differentiation is also possible between populations without primary divergence of male color patterns. *Pt.* 'orange chest' which possesses intermediate and highly variable jaw shape also has the widest range of depth distributions of any species surveyed during this study (Ribbink *et al.* 1983). Populations of *Pt.* 'orange chest' from Zimbawe Rock and Domwe Island and populations of *Pt.* 'intermediate' from Otter Island and Thumbi West Island showed significant differentiation along PC1 (Table 5). Cichlid communities are made of multispecies guilds and are shaped by a complex tapestry of microhabitat features and behavioral interactions (Hori 1997, Kohda 1998), with each location differing in species composition and density

101

(Ribbink *et al.* 1983). Therefore it should not be surprising that populations of cichlids in isolated habitats would experience novel ecological selective pressures that lead to character displacement of morphological characters observed in other circumscribed vertebrate communities (Schluter & McPhail 1993, Losos 2000). The elucidation of the relationships between recently evolved taxa is a critical first step in unraveling the forces acting during speciation. The parallel and rapid evolution of trophic morphology, habitat preference and male color pattern suggest that while sexual selection is an ubiquitous force acting during recent cladogenic events, natural selection continues to act both independently and synergistically during the formation of new species in this extraordinary microcosm of vertebrate speciation.

Table 4.1

Taxa of the subgenus *Pseudotropheus tropheops* sampled for morphological and AFLP fingerprint analysis.

Species	Location	Sample Size		Habitat Preference	
		AFLP	Morphology	Depth	Sediment
T. 'Black Dorsal'	West Reef	3	3	Deep	Poor
T. 'Broadmouth'	Otter Island	3	3	Shallow	Rich
T. 'Chinyamwezi'	Chinyamwezi Island	3	19	Shallow	Poor
T. 'Chinyankwazi'	Chinyankwazi Island	3	17	Shallow	Poor
T. 'Gome yellow'	Chiofu	3	4	Shallow	Unknown
T. gracilior	Domwe Island	3	32	Deep	Rich
T. gracilior	Otter Island	3	8	Deep	Rich
T. 'Intermediate'	Thumbi West Island	3	35	Deep	Rich
T. 'Intermediate'	Otter Island	3	5	Deep	Rich
T. 'Intermediate'	Mazinzi Reef	3	6	Deep	Rich
T. 'Lilac'	Thumbi West Island	2	21	Shallow	Poor
T. 'Lilac'	Otter Point	3	16	Shallow	Poor
T. microstoma	Domwe Island	3	0	Deep	Rich
T. microstoma	Otter Point	3	25	Deep	Rich
T. 'Mumbo lilac'	Mumbo Island	3	37	Shallow	Poor
T. 'Mumbo zebra'	Mumbo Island	3	8	Deep	Rich
T. 'Orange chest'	Otter Island	3	0	Shallow	Poor
T. 'Orange chest'	Thumbi West Island	3	26	Shallow	Poor
T. 'Orange chest'	Domwe Island	0	30	Shallow	Poor
T. 'Orange chest'	Zimbawe Rock	0	22	Shallow	Poor
T. 'Otter gold'	Otter Island	3	5	Shallow	Poor
T. 'Red cheek'	Thumbi West Island	3	31	Shallow	Poor
T. 'White dorsal'	Namtumbi	3	1	Shallow	Unknown
T. 'Yellow gular'	Chiofu	3	3	Shallow	Rich
T. 'Yellow gular'	Namtumbi	3	0	Shallow	Rich

103

Table 4.2

Number of phylogenetic characters generated per selective PCR amplification.

Primer Pair	Number of Characters		Uninformative	
	Total	Informative	Variable	Constant
ACACAA	471	343	50	78
ACACAG	386	247	62	77
ACACTA	424	335	55	34
ACACTT	451	349	60	42
ACCCAA	483	366	72	45
ACCCAC	422	310	60	52
ACCCAT	468	396	54	18
ACCCTA	458	355	68	35
ACTCAG	448	323	65	61
ACTCAT	474	338	59	77
ACTCTA	425	342	48	35
ACTCTG	343	247	52	44
ACTCTT	431	304	57	70
AGCCAG	392	227	62	103
AGCCAT	483	371	84	28
AGCCTA	480	401	53	26
AGGCTG	425	281	72	72
AGGCTT	489	369	64	56
Total=	7953	5905	1097	951
Mean =	441.8	328.0	60.9	52.9

104

Table 4.3

Probabilities that specific color states define a monophyletic lineage within the *Pseudotropheus tropheops* species complex. Probabilities were generated by comparing constrained and unconstrained trees using Shimodaira & Hasegawa tests (Shimodaira & Hasegawa 1999).

Color State	Male Color Pattern Characters								
	Chin	Shoulder	Dorsalateral Band	Chest	Midlateral Band	Caudal Peduncle	Dorsal Fin	Pelvic Fin	Anal Fin
Yellow	<0.0001	<0.0001	NA	0.0009	<0.0001	<0.0001	<0.0001	0.0065	NA
Orange	0.0002	<0.0001	0.1189	<0.0001	<0.0001	0.0254	NA	0.0016	NA
White	NA	NA	NA	NA	NA	NA	NA	NA	0.0005
Blue	0.5726	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7698	<0.0001
Grey	NA	NA	0.0005	NA	0.0006	0.2228	0.9217	0.0103	0.3323
Black	<0.0001	0.0151	NA	0.0002	0.6754	0.6556	NA	<0.0001	0.0019
Brown	NA	NA	0.7000	NA	NA	0.4828	NA	NA	NA
Blue/Yellow mixed	NA	NA	NA	NA	NA	NA	<0.0001	0.9093	NA
# of Changes	7	11	7	7	10	8	10	9	10

105

Table 4.4

Mean pair-wise differences between species from a Tukey's test of principle component variables (PC1 & PC2) of lower lateral jaw morphology. Numbers in bold indicate significant differentiation of jaw shape ($p<0.05$).

PC1	BLD	BRM	CHK	CHW	GRC	INT	LIL	LLM	MIC	MMZ	ORC	OTG	RCH
Black Dorsal'	0.000												
Broadmouth'	0.000	0.000											
Chinyankwazi'	0.035	0.036	0.000										
Chinyamwezi'	0.034	0.035	-0.001	0.000									
gracilior	**-0.074**	**-0.074**	**-0.109**	**-0.109**	0.000								
Intermediate'	**-0.052**	-0.052	**-0.087**	**-0.087**	0.022	0.000							
Lilac'	-0.007	-0.007	**-0.043**	-0.042	**0.067**	**0.045**	0.000						
Lilac Mumbo'	0.017	0.017	-0.019	-0.018	**0.091**	**0.069**	0.024	0.000					
microstoma	**-0.077**	**-0.076**	**-0.112**	**-0.111**	-0.003	-0.024	**-0.069**	**-0.093**	0.000				
Mumbo Zebra'	**-0.098**	**-0.098**	**-0.133**	**-0.133**	-0.024	**-0.046**	**-0.091**	**-0.115**	-0.022	0.000			
Orange Chest'	-0.012	-0.011	**-0.047**	**-0.046**	**0.062**	**0.040**	-0.004	**-0.028**	**0.065**	**0.087**	0.000		
Otter Gold'	**-0.071**	**-0.070**	**-0.106**	**-0.105**	0.003	-0.018	**-0.063**	**-0.087**	0.006	0.028	**-0.059**	0.000	
Red Cheek'	-0.008	-0.007	**-0.043**	-0.042	**0.066**	**0.044**	0.000	-0.024	**0.069**	**0.091**	0.004	**0.063**	0.000

PC2	BLD	BRM	CHK	CHW	GRC	INT	LIL	LLM	MIC	MMZ	ORC	OTG	RCH
Black Dorsal'	0.000												
Broadmouth'	-0.041	0.000											
Chinyankwazi'	-0.039	0.003	0.000										
Chinyamwezi'	-0.045	-0.003	-0.006	0.000									
gracilior	**-0.069**	-0.028	-0.030	-0.025	0.000								
Intermediate'	-0.042	-0.001	-0.003	0.002	0.027	0.000							
Lilac'	-0.034	0.008	0.005	0.011	**0.035**	0.008	0.000						
Lilac Mumbo'	**-0.087**	-0.046	**-0.048**	**-0.043**	-0.018	**-0.045**	-0.053	0.000					
microstoma	-0.031	0.010	0.008	0.013	**0.038**	0.011	0.003	**0.056**	0.000				
Mumbo Zebra'	-0.051	-0.009	-0.012	-0.006	0.018	-0.009	-0.017	0.036	-0.020	0.000			
Orange Chest'	**-0.046**	-0.004	-0.007	-0.001	0.024	-0.003	-0.012	**0.042**	-0.014	0.005	0.000		
Otter Gold'	-0.037	0.004	0.002	0.008	0.032	0.005	-0.003	0.050	-0.006	0.014	0.008	0.000	
Red Cheek'	-0.047	-0.005	-0.008	-0.002	0.022	-0.005	-0.013	**0.040**	-0.016	0.004	-0.001	-0.010	0.000

Table 4.5

Mean pair-wise differences between replicated populations of the same species from a Tukey's test of principle component variables (PC1) of lower lateral jaw morphology. Numbers in bold indicate significant differentiation of jaw shape ($p < 0.05$).

PC1	GRA (DI)	GRA (OP)	INT (MZ)	INT (OI)	INT (TW)	LIL (OP)	LIL (TW)	MMZ	ORC (DI)	ORC (TW)	ORC (ZR)
gracilior (DI)	0.000										
gracilior (OP)	0.003	0.000									
Intermediate' (MZ)	0.039	0.036	0.000								
Intermediate' (OI)	0.068	0.065	0.029	0.000							
Intermediate' (TW)	0.009	0.006	-0.030	**-0.059**	0.000						
Lilac' (OP)	0.048	0.045	0.009	-0.020	0.039	0.000					
Lilac' (TW)	0.094	0.092	0.055	0.026	0.085	0.047	0.000				
Mumbo Zebra'	-0.023	-0.025	-0.062	**-0.091**	-0.032	-0.070	-0.117	0.000			
Orange Chest' (DI)	0.049	0.046	0.010	-0.019	0.040	0.001	-0.046	0.071	0.000		
Orange Chest' (TW)	0.064	0.062	0.025	-0.004	0.055	0.017	-0.030	0.087	0.016	0.000	
Orange Chest' (ZR)	0.087	0.084	0.048	0.019	0.078	0.039	-0.007	0.110	**0.038**	0.023	0.000

107

Figure 4.1
Sample locations along the southeast arm of Lake Malawi, Africa.

108
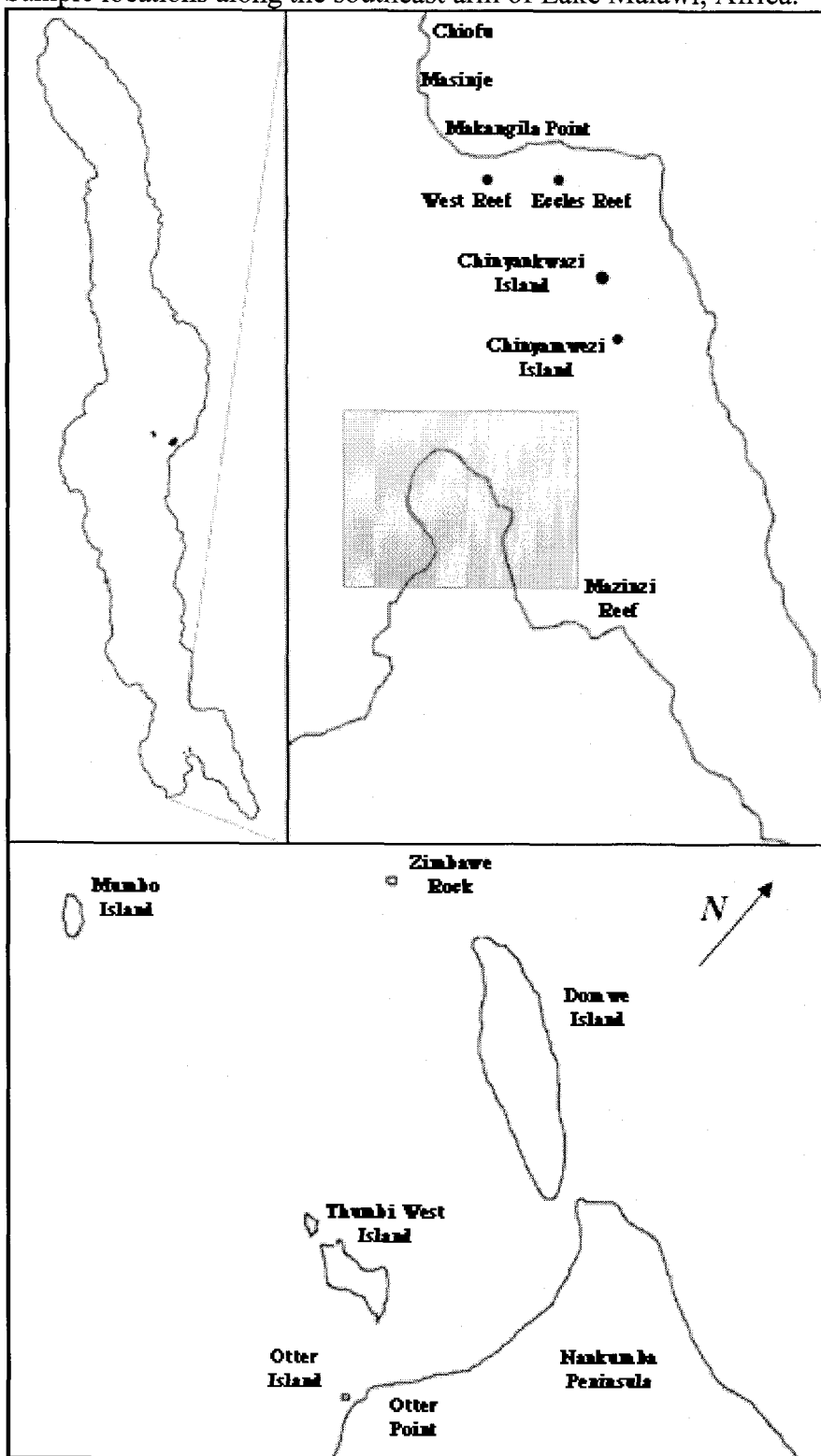
Figure 4.2

(a.) Landmark positions on the lower oral jaw included the rostral tip of the dentary, tip of the rostral process of the articular, dorsal tip of the dentray process, dorsal tip of the articular process, dorsal process of the suspensoriad articulation facet, postarticulation process, retroarticular process, and the rostral process of the coulter area (after Barel et al. 1976). (b.) Male color characters scored as black, white, orange, yellow, blue, grey, and brown (after Ribbink et al. 1983).

109
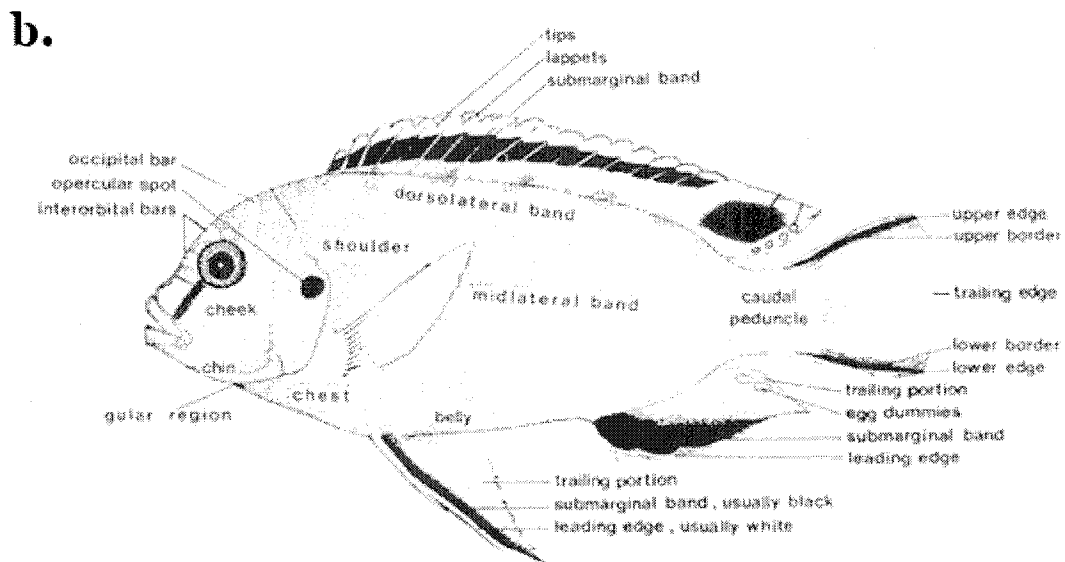
Figure 4.3

Phylogram of the subgenus *Pseudotropheus tropheops* from the southeast arm of Lake Malawi based on Nei Li's genetic distance and constructed using the Neighbor Joining algorithm. Bars on the right indicate the current generic assignment of each taxon.
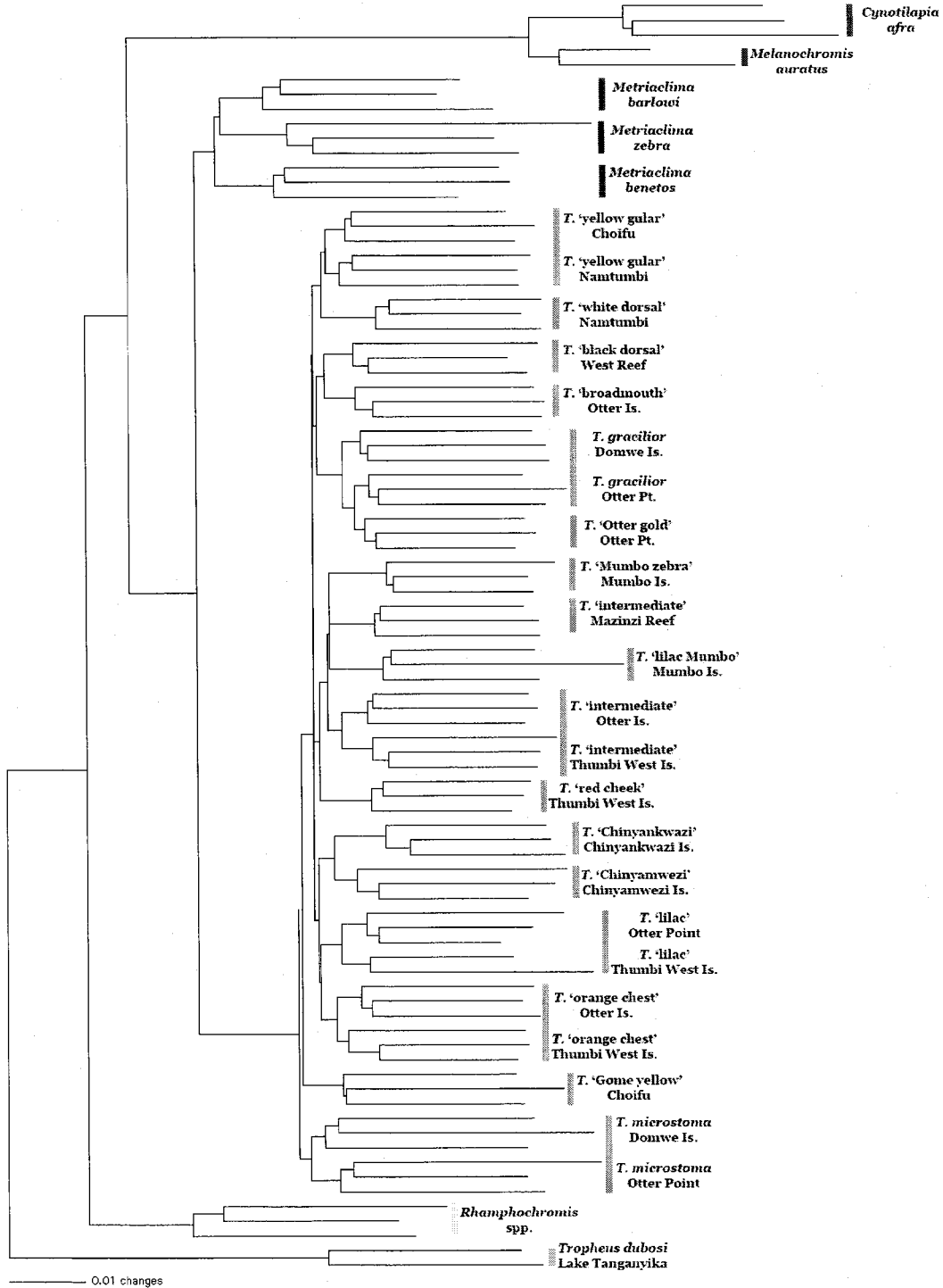
Figure 4.4
Phylogeny of subgenus *Pseudotropheus tropheops* from the southeast arm of Lake Malawi based on Nei Li's genetic distance and constructed using the Neighbor Joining algorithm. Numbers at each node indicate the bootstrap values (from 1,000 replicates) for that node. Bars on the right indicate the current generic assignment of each taxon.

111
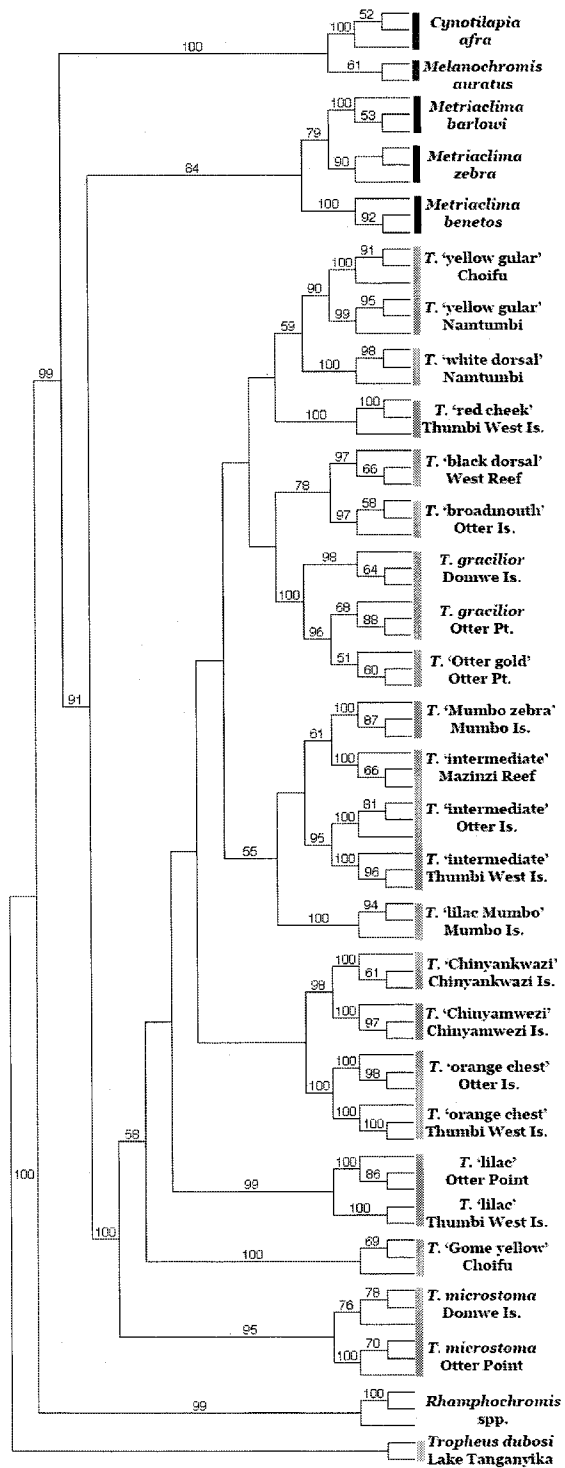
Figure 4.5.

Parallel evolution of male color characters during the diversification of the *Pseudotropheus tropheops* species complex. Numbers at each node indicate the bootstrap values (from 1,000 replicates) for that node.
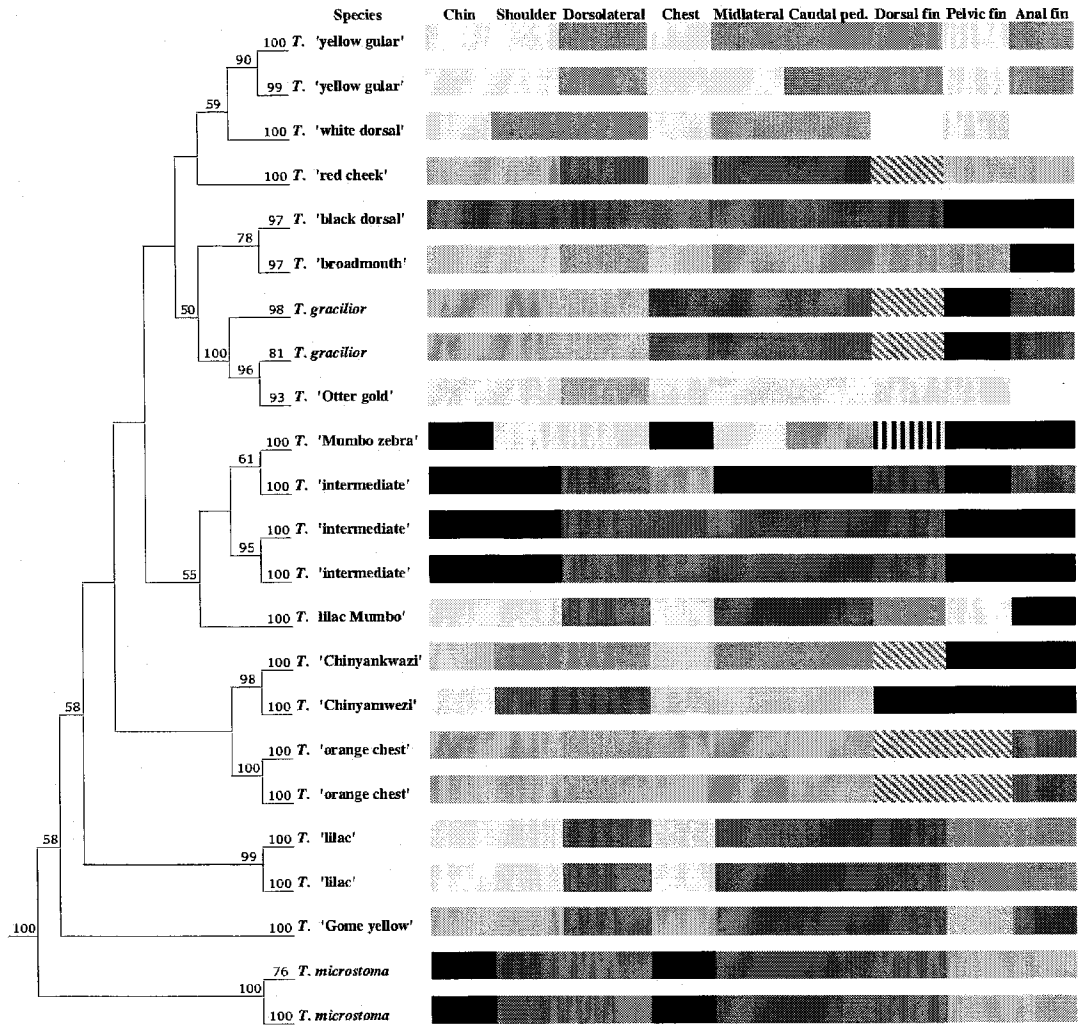
Figure 4.6

Parsimony reconstruction of the number of unambiguous color changes along each branch during the diversification of the *Pseudotropheus tropheops* species complex.

T. 'Yellow Gular'

T. 'White Dorsal'

T. 'Red Cheek'

T. 'Black Dorsal'

T. 'Broadmouth'

T. gracilior

T. 'Otter Gold'

T. 'Intermediate'

T. 'Mumbo Zebra'

T. 'Intermediate'

T. 'Lilac Mumbo'

T. 'Orange Chest'

T. 'Chinyankwazi'

T. 'Chinyamwezi'

T. 'Lilac'

T. 'Gome Yellow'

T. microstoma

#Chars. that change
Unambiguously on branch

0
1
2
3
4
5
6
7
8
9 or more

113
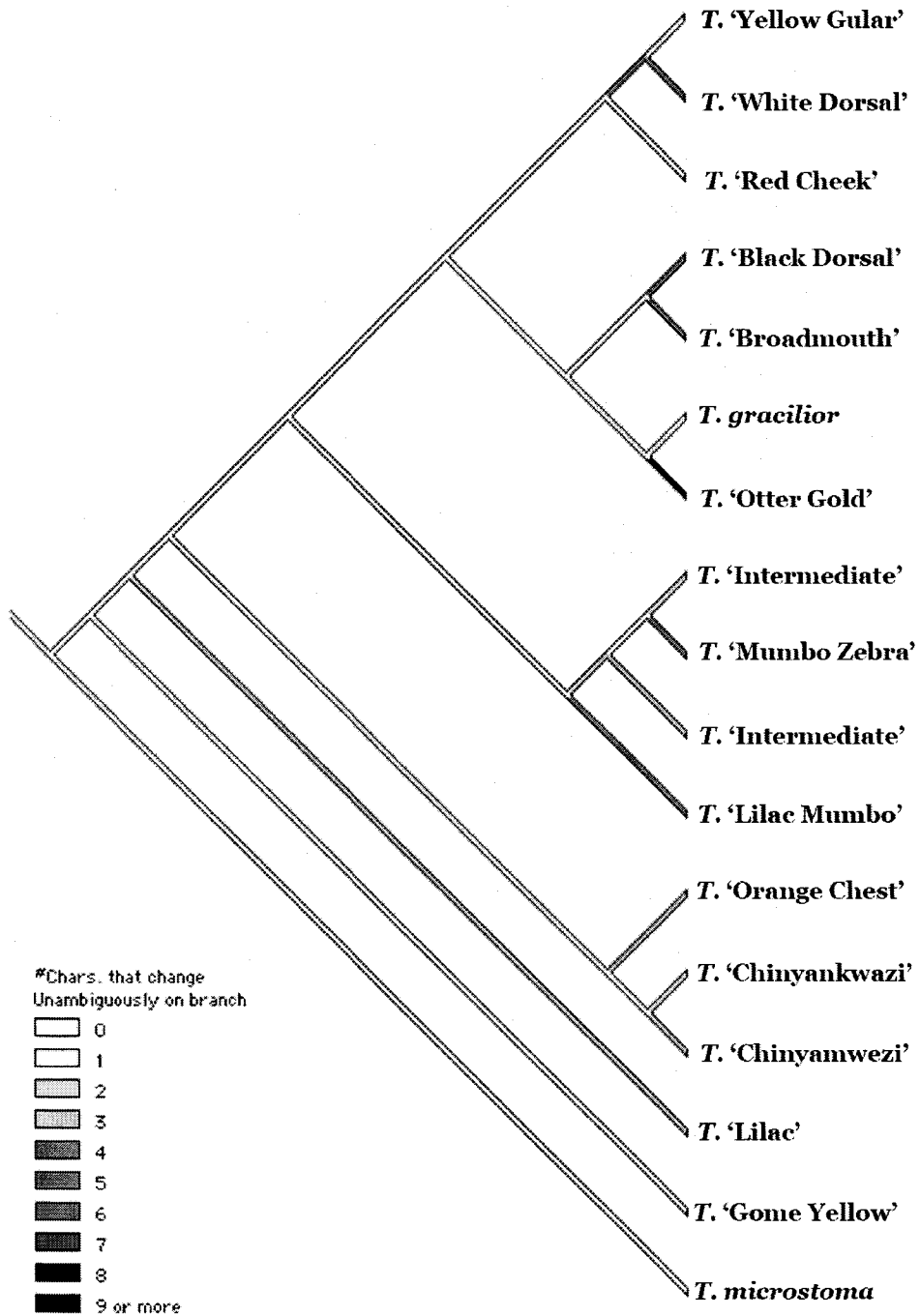
Figure 4.7

(a.) Variation in jaw shape within the *Pseudotropheus tropheops* species complex along the first two principle component axes. A multivariate analysis of variance identified significant differences in jaw shape among species (MANOVA, Wilks' $\lambda=0.2637$, $F_{12,212}=16.65$, $p<0.0001$) along principle components (PC)1 and 2, which explained 39% and 20% of the variation respectively (b.) Variation in jaw shape along PC1 is strongly associated with species depth distribution. Species that prefer shallow habitats (less than 10m, green) tend to have jaws with higher mechanical advantage and are strongly differentiated along PC1 (ANOVA, $F_{1,220}= 191.023$, $p<0.00001$) from species that prefer deeper habitats (greater than 10m, blue) and possess jaws with lower mechanical advantage.
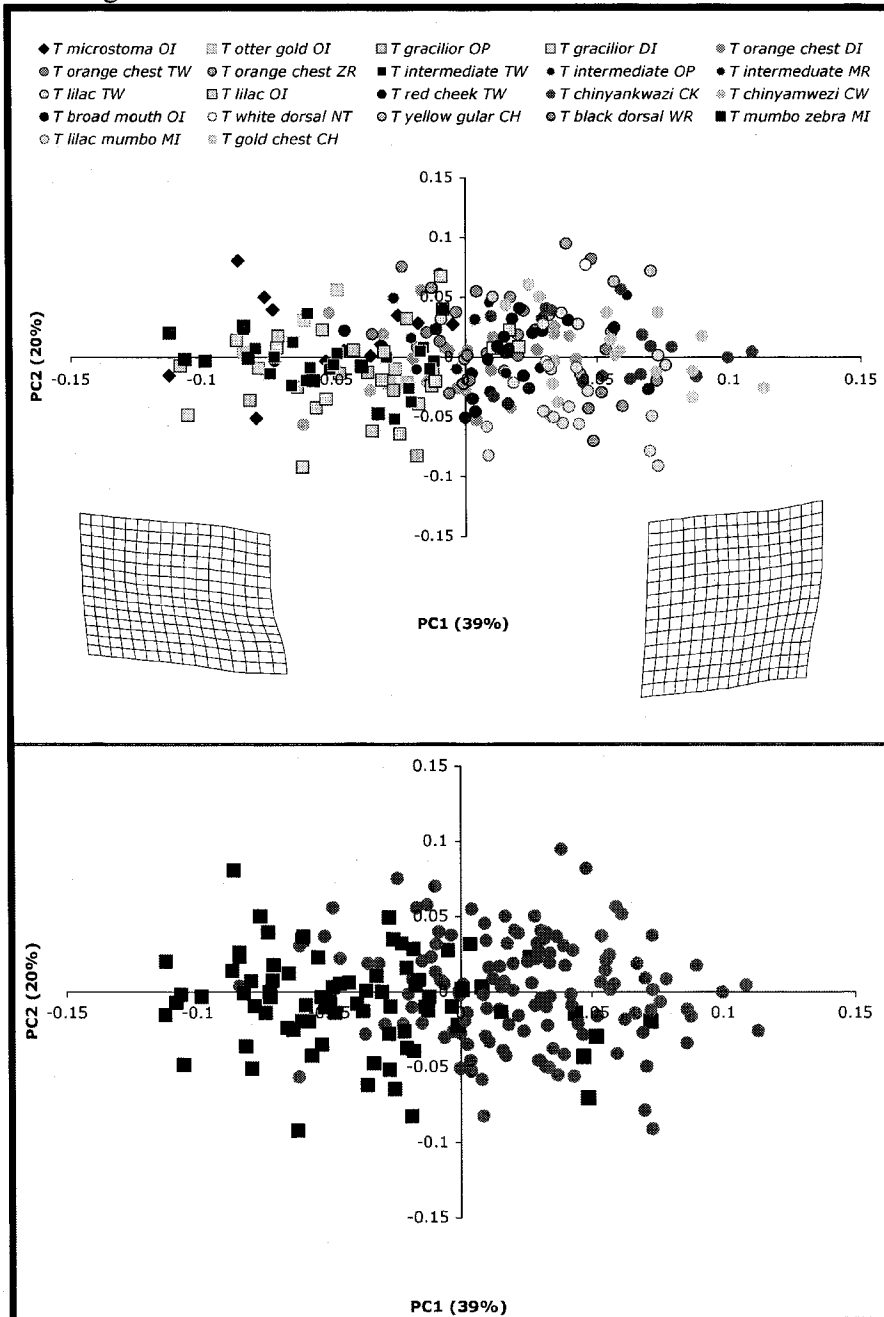
Figure 4.8

Parsimony reconstruction of depth preference. Neither shallow dwelling (less than 10m, green) nor deep dwelling (greater than 10m, blue) taxa define a monophyletic lineage (SH p<0.0001 for either deep or shallow habitats) indicating the parallel evolution of habitat preference during the diversification of the *Pseudotropheus tropheops* species complex. (above or below 10m).
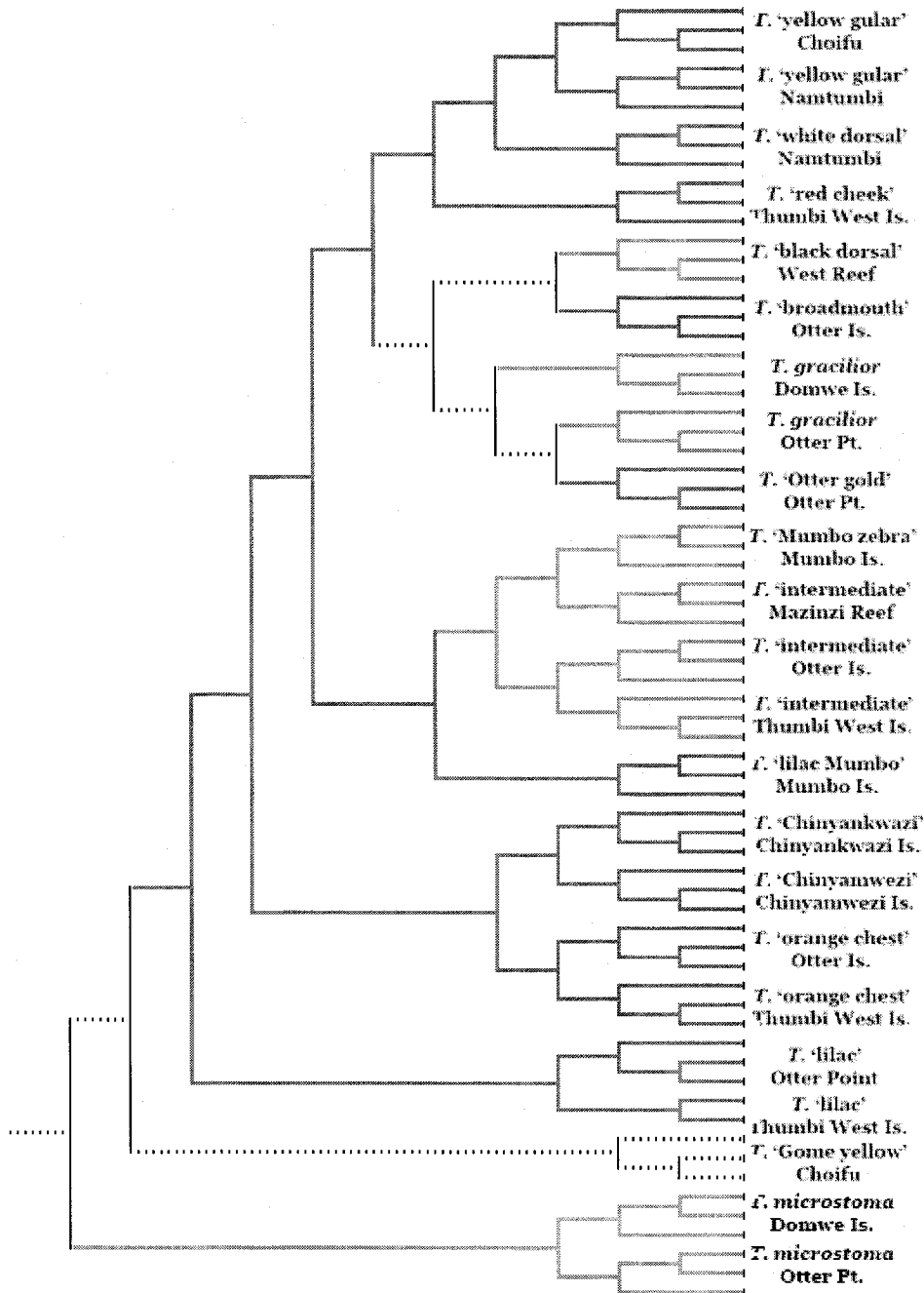
CHAPTER 5

AXES OF DIFFERENTIATION IN THE

BOWER BUILDING CICHLIDS OF LAKE MALAWI

Abstract

The 500-1000 cichlid species endemic to Lake Malawi constitute one of the most rapid and extensive radiations of vertebrates known. There is a growing debate over the role natural and sexual selection have played in creating this remarkable assemblage of species. Phylogenetic analysis of the Lake Malawi species flock has been confounded by the lack of appropriate morphological characters and an exceptional rate of speciation, which has allowed ancestral molecular polymorphisms to persist within species. To overcome this problem I used Amplified Fragment Length Polymorphism (AFLP) to reconstruct the evolution of species within three genera of Lake Malawi sand dwelling cichlids that construct elaborate male display platforms, or bowers. Sister taxa with distinct bower morphologies, and that exist in discrete leks separated by only 1-2m of depth, are divergent in both sexually selected and ecological traits. Our phylogeny suggests that the forces of sexual and ecological selection are intertwined during the speciation of this group and specific bower characteristics and trophic morphologies have evolved repeatedly. These results suggest that trophic morphology and bower form may be inappropriate characters for delineating taxonomic lineages. Specifically the morphological characters used to describe the genera *Lethrinops* and *Tramitichromis* do not define monophyletic clades. Using a

116

combination of behavioral and genetic characters I were able to identify several cryptic cichlid species on a single beach, which suggests that sand dweller species richness has been severely underestimated.

Introduction

Traditional allopatric models of speciation begin with the disruption of gene flow by geographic barriers, which allows separate populations to be pushed into novel evolutionary trajectories (Mayr 1963). Recent discussion has focused on the relative importance of natural versus sexual selection during population divergence (van Doorn *et al.* 1998, Danley & Kocher 2001, Streelman & Danley 2003, Kirkpatrick & Nuismer 2004, Arnegard & Kondrashov 2004, Coyne & Orr 2004). In ecological models, resource competition leads to genetic divergence through disruptive natural selection (Orr & Smith 1998, Rundle & Nosil 2005). If the populations are allopatric, then reproductive isolation is established as a by-product of ecological character divergence, or by genetic drift. Sexual selection can increase the rate of divergence among such populations (West-Eberhard 1983). Several models suggest that speciation can be driven by sexual selection alone (Lande 1981, Wu 1985, Turner & Burrows 1995, Higashi *et al.* 1999, Lande *et al.* 2001). Since sexual selection acts on mate recognition systems, it has the potential to create reproductive isolation without ecological differentiation (Panhuis *et al.* 2001). Divergence of courtship signals is a characteristic common to many species rich lineages (Barraclough *et al.* 1995, Boughman 2002, Streelman & Danley 2003). However, some authors contend that without subsequent niche divergence, competitive exclusion would lead to the extinction of one or both sister taxa during secondary contact (van Doorn *et al.* 1998, Arnegard & Kondrashov 2004).

The extraordinary radiation of Lake Malawi's estimated 1,000 species of endemic cichlid fishes has become a model system for studying adaptive radiation (Kornfield &Smith 2000) and

118

gives us an opportunity to identify the forces shaping recently evolved sister taxa. Reconstructing the phylogenetic history of this rapid radiation has proved challenging, but the analysis of large numbers of independent genetic loci using AFLP has allowed significant progress to be made within the clade of rock-dwelling cichlids (Albertson *et al.* 1999, Allender *et al.* 2003). The patterns of divergence revealed by these studies encouraged Danley and Kocher (2001) to suggest that natural and sexual selection have acted in a sequential pattern of 3 stages during the diversification of this species rich lineage. The initial stage of radiation is characterized by adaptation to distinct macrohabitats and produced the major lineages which contain most of the current species diversity in the lake (the rock dwelling *mbuna* and the sand dwelling *utaka* and *chisawasawa*). The reciprocal monophyly of the rock- and sand-dwelling lineages has been confirmed by data from mtDNA (Moran *et al.* 1994, Kocher *et al.* 1995, Salzburger *et al.* 2002) and SINE insertions (Takahashi *et al.* 2001). The second stage of radiation was driven by competition for trophic resources. AFLP data confirm the monophyly of rock-dwelling genera (Albertson *et al.* 1999) that were originally defined largely by their feeding morphology (Regan 1922, Trewavas 1935). The third stage of radiation in the rock-dwelling species is characterized by the divergence of secondary male colour patterns without dramatic changes in trophic morphology (Danley & Kocher 2001). Closely related species often differ in color pattern (Allender *et al.* 2003), which has been shown to be an important component of conspecific mate recognition (Seehausen & van Alphen 1998, Kidd *et al.* in press). While this radiation-in-stages model was initially proposed to explain diversification of the *mbuna*, it has been extended to explain species diversity in other well-characterized vertebrate radiations (Streelman & Danley 2003).

Sister group comparisons are one of the best ways to identify the factors involved in the evolution of diversity (Barraclough *et al.* 1998) and the sand-dwelling lineage of Lake Malawi cichlids affords us the opportunity for such a comparison. Like their *mbuna* cousins, the sand dwelling genera are defined primarily by trophic morphology (Eccles & Trewavas 1989). Unlike the *mbuna*, the nuptial coloration of the sand dwelling males is relatively inconspicuous (Turner 1996, McKaye *et al.* 2001). However, they do construct elaborate bowers of sand and gravel from which they display to potential mates (McKaye 1991, McKaye *et al.* 2001). These bowers are constructed in heterospecific leks in which each species builds a stylized bower with a species-specific shape (McKaye 1983). All endemic Lake Malawi cichlids are maternal mouthbrooders, so these bowers are used for male display and egg laying purposes only. After spawning the female leaves the male's territory with the fertilized eggs in her buccal cavity.

McKaye (1991) described 10 categories of qualitatively different bower form in Lake Malawi cichlids, including pits, mounds and volcanos. Bowers within each category vary quantitatively, ranging in size from a few centimeters to 3m across (Stauffer *et al.* 1995, McKaye *et al.* 2001). If a male takes over a heterospecific bower, he will initially display over the existing bower while gradually altering its form and structure to his species-specific style (McKaye 1984). Bower shape is so species-specific that Stauffer *et al.* (1993) used it as a taxonomic character to distinguish three new species (*Copadichromis conophoros*, *C. cyclicos*, and *C. thinos*).

The development of robust phylogenies for the sand-dwelling lineage has been problematic (Kornfield & Smith 2000, Turner *et al.* 2001). Phylogenies based on morphology are limited by the lack of appropriate morphological synapomorphies (Stiassny 1991). Convergence of morphological characters is seen among lineages within and between rift valley

120

lakes (Kocher *et al.* 1993, Reinthal & Meyer 1997). The construction of robust phylogenies

from most DNA sequence data has been confounded by the extraordinary tempo of cichlid

evolution has allowed the retention of genetic polymorphisms (Moran & Kornfield 1993, Parker

& Kornfield 1997). The problem of incomplete lineage sorting can be overcome by surveying

thousands of independent restriction sites spread across the nuclear genome using Amplified

Fragment Length Polymorphisms (AFLP) (Albertson *et al.* 1999, Seehausen *et al.* 2003,

Allender *et al.* 2003). Here I used AFLP markers to reconstruct the evolutionary history of three

bower building genera: *Copadichromis*, *Protomelas* and *Lethrinops* (including *Tramitichromis*).

Our goal was to identify groups of recently evolved sister taxa which might provide information

about the mechanisms of speciation in this group. I also wanted to evaluate instances of

morphological convergence by comparing the current morphology-based taxonomy to an

independent molecular phylogeny. Finally, I wished to discover patterns in the evolution of

bower shape and melanin pigmentation patterns within the context of a robust phylogeny for

members of the sand-dwelling clade in Lake Malawi.

Methods

Taxa Studied

Individuals of the genera *Copadichromis*, *Protomelas* and *Lethrinops* were collected for this study (Table 1). The genus *Copadichromis* consists of species with elongated premaxillary pedicels that are extended forward to form a protrusible mouth. Additionally, these species have weak jaws and teeth that are recurved simple or bicuspid (Eccles & Trewavas 1989). Eccles and Trewavas (1989) described the genus *Protomelas* without defining any synapomorphies common to the group. Species of this genus lack any of the synapomorphies used to define other sand-dwelling genera, but do share one or two longitudinal dark bands that may be overlaid on vertical bars. The genus *Lethrinops*, as established by Regan (1922) and revised by Trewavas (1931), is separated from other haplochromine genera based on oral jaw dentition. Eccles and Trewavas (1989) described two new genera (*Tramitichromis* and *Taeniolethrinops*), which share the *Lethrinops* dentition.

A nested set of outgroups was also included in this study. *Metriaclima zebra* is a member of the rock-dwelling clade that is sister to the sand-dwellers. *Rhamphochromis* is a more basal lineage in the Lake Malawi radiation (Moran *et al.* 1994, Kocher *et al.* 1995). *Tropheus duboisi* is part of Lake Tanganyika's haplochromine lineage, which is sister to both the Lake Malawi and Lake Victoria species flocks (Meyer 1993).

Sample Collection and Field Work

All samples were taken from live fish collected in the field, with the exception of *Tropheus duboisi*, which were purchased through the aquarium trade. Field collections were

122

made in Lake Malawi National Park on the Nankumba Peninsula (Fig. 1a) during July and August 2001. Chembe beach is a 4km sandy beach that stretches from Domwe Island to Otter Point (Lewis *et al.* 1986). The substrate alternates between fine sand, gravel, and flocculent mud. Beds of *Vallisneria* and *Potamogeton* punctuate areas of sand. Black Rock Beach is a narrow (5m long) beach of fine sand wedged between continuous rocky coastlines. The sand/rock interface is very shallow throughout this area (about 1m) and underwater the narrow beach opens to a wide expanse of open sand punctuated with beds of weeds. The Songwe Hill collection site is on the eastern side of the Nankumba Peninsula and is described in Ribbink *et al.* (1983). Initial underwater surveys were performed to identify populations that differed in bower form (Fig. 2a-d). The following measurements were taken for each individual bower (n=5-30): platform diameter, base diameter, total height, lip height, and slope (Fig. 2e). Bower measurements were recorded from every population sampled with the exception of *Tramitichromis* 'microstoma type.' Populations that differed in bower design were treated as isolated breeding populations, regardless of overlapping morphological characters. Prior to collection, each male was observed to verify that he was actively maintaining and defending the bower he occupied. Individual males were surrounded with a monofilament net, which was slowly drawn closed while maintaining visual contact with the specimen. This ensured that the appropriate male was captured for each bower type. Fin clips were taken from the right pectoral fin of each specimen and stored in 95% EtOH. Three to five voucher specimens were preserved in 10% formalin and later transferred to 70% ethanol for long-term storage.

Morphology

Additional specimens (3-50) were collected and dried on site. These were later disarticulated in the lab using a colony of dermestid beetles. Digital photographs of the lower pharyngeal and oral jaws were taken with a SPOT digital camera (Diagnostic Instruments, Inc.) mounted on a Zeiss SV11 dissecting scope. The photographs were imported into NIH Image 1.59 and analyzed following the methods of Albertson and Kocher (2001). All specimens were examined for the haplochromine vs. *Lethrinops* oral dentition and the presence of the 'keel' on the lower pharyngeal jaw. Length and width of the lower pharyngeal bone and the muscular process (horn width) were measured following Barel *et al.* (1977) and Hoogerhoud (1984). The tooth area and proportion of molariform teeth was calculated following Barel *et al.* (1977).

Molecular Techniques

Genomic DNA was extracted from the fin clips of 3 individuals of each taxa (2 for *Protomelas similis* and *Lethrinops parvidens*) using a standard phenol/chloroform extraction (Wang *et al.* 2003). AFLP is a DNA fingerprinting technique which characterizes thousands of restriction polymorphisms spread throughout the genome (Vos *et al.* 1995). In this method, genomic DNA is first double-digested using two restriction enzymes (in this case EcoRI and MseI). Double stranded adapters are then ligated onto the "sticky" ends of the fragments. An initial "preselective" PCR reaction amplifies a subset of fragments that match adapter primers containing an additional nucleotide (EcoRI-A and MseI-C). This product of this pre-selective amplification was then used as the template for 11 different "selective" amplifications performed with primers containing an additional 2 nucleotide extension (E-AGG, M-CTT, M-CTG; E-ACT, M-CTA, M-CAG, MCAT; E-ACA, M-CAA, M-CAG; E-AGC, M-CAG, M-CAT; E-ACC, M-CAA, M-CAC). The result of these amplifications is a PCR product containing a small subset of

124

the genomic fragments produced by the initial restriction. The detailed protocols can be viewed at (http://hcgs.unh.edu/protocol/basic).

Fragments were separated using a Beckman Coulter CEQ 8000 capillary sequencer. Since only the EcoRI selective primers were fluorescently labeled, only those fragments containing an EcoRI site were detected. Peaks were scored using a quartic model with a slope threshold of 2.0% and relative peak height of 5.0%. Bands were scored as present/absent using Beckman Coulter's Fragment Analysis Module. The presence of each fragment was confirmed manually. Fragments between 70-600 bp in size were binned (1 nucleotide bin width) using Beckman Coulter's AFLP Analysis Software. The binary output was imported to an Excel spreadsheet and formatted for PAUP 4.0b8 (Swofford 2001).

Phylogenetic Analysis

Matrices were generated by calculating Nei and Li's (1979) genetic distance. The phylogram was constructed from 1,000 bootstrap replicates using a neighbor joining algorithm in PAUP v.4.0b8 (Swofford 2001). The data were tested for hierarchical structure by analyzing the frequency and distribution of tree lengths for 1,000,000 randomly generated trees (Hillis & Huelsenbeck 1992). Kishino-Hasegawa tests (KH) were used to assess the validity of alternate topological hypotheses (Kishino & Hasegawa 1989). We tested the reciprical monophyly of specific sister taxa pairs, by constructing a suite of contraint trees in PAUP v.4.0b8 which sequentially forced the exclusion of each individual from the clade. Using the same parameters described above, PAUP identified the best tree that included the constraint and a KH test was used to compare the constrained and unconstrained trees (n=12-36 permutations for each clade). The maximum p-value is reported for each node.

Because hybridization may occur between taxa, the traditional phylogenetic view of a bifurcating tree may not be an accurate representation of the evolutionary history during rapid adaptive radiations (Posada & Crandall 2001, Seehausen 2004). Two methods were employed to detect the effect of reticulation during the diversification of these bower-building taxa. The first method was the homoplasy excess test described by Seehausen (2004) and utilized by Schliewen & Klee (2004). Since hybrids are intermediate to both parents, hybrid taxa will increase the amount of homoplasy within the data set, which can produce identifiable conflict within the clades contributing to the hybridization. The removal of excess homoplasy in the data set (by removing hybrid taxa) will result in a substantial increase in the resolution of the parental clades. Following Schliewen and Klee (2004), each taxa was sequentially removed and the phylogeny reconstructed using the same parameters described above. Bootstrap values for each node of each pruned tree were recorded and compared. Additionally, the pairwise distance matrix created in PAUP was imported into SplitsTree v.2.4 (Hudson 1998). We used split decomposition (Bandelt & Dress 1992) to produce a 'splits graph' which displays conflicting topologies as a network. For ideal data, this method will produce a standard bifurcating tree, however less ideal data gives rise to a tree-like network. Cassens *et al.* (2003) found that split decomposition revealed network topology for a data set that yielded unambiguous topology when analyzed with either statistical parsimony or median joining algorithms.

126

Results

AFLP Phylogeny

Eleven primer pair combinations generated 4,355 characters ($\overline{X}$=397.8 per primer pair).

Of these, 3,171 were polymorphic ($\overline{X}$=288.3 per primer pair). A plot of the length of 1,000,000

random trees demonstrated significant non-random structure to the data set (g_1= -0.536, 69

samples, p<0.01). These data were used to construct a distance tree (Fig. 3) with all but one

node resolved above a 50% bootstrap value. The entire tree has a mean bootstrap value of 86.7%

(Fig. 4). Replicate samples from the same population cluster together with a mean bootstrap

value of 97.3%. Species collected from multiple locations (*Lethrinops auritus*, *Metriaclima*

zebra) also cluster together consistently.

This tree recovers the expected relationship of all outgroup taxa. *Tropheops duboisi* from

Lake Tanganyika was used to root the tree. The genus *Rhamphochromis* emerges as basal to all

other Lake Malawi samples, which corresponds to previous mtDNA based (Kocher *et al.* 1993,

Meyer 1993) and AFLP based (Albertson *et al.* 1999, Allender *et al.* 2003, Seehausen *et al.*

2003) phylogenies. The mbuna clade, represented by *Metriaclima zebra*, is sister to all of the

sand dweller taxa sampled.

The species of *Copadichromis* sampled in this study form a monophyletic lineage (KH

p<0.0002). *Protomelas* 'black rock', *P. taeniolatus* and *P. similis* also form a monophyletic

clade (KH p=0.022). The genera *Copadichromis*, *Protomelas* and *Taeniolethrinops* are

embedded within a clade that also contains multiple representatives of *Lethrinops* and

Tramitichromis. Neither the *Lethrinops* oral dentition, nor the *Tramitichromis* pharyngeal keel,

appear to define monophyletic clades (KH p<0.0001 for both alternatives).

127

Both tests used to detect the presence of conflicting phylogentic signal attributed to hybridization yielded similar results. The removal of both populations of *Lethrinops auritus* substantially increased the bootstrap support value for the node uniting *L. lethrinus* and *L. parvidens* (Fig. 5a). The removal of all other taxa failed to produce any outlying improvement for the bootstrap support value of any other node. Similarly, split decomposition also indicated potential reticulation within this lineage. There was no evidence of network-like relationships when all taxa were used to construct the splits graph. However, subdividing the data set to focus on specific lineages (*Copadichromis. Protomelas*, *Lethrinops* and *Tramitichromis*) revealed that a conflicting relationship exists between *L. lethrinus* and *L. auritus* of Songwe Hill (Fig. 5b).

Copadichromis

When erecting the genus *Copadichromis*, Eccles and Trewavas (1989) considered oral jaw structure to be more important than melanin pattern. Species with different colour patterns (barred, 3 spot, and mid-lateral striped) were unified by a synapomorphic protractile mouth. While several authors have suggested that this genus is polyphyletic (Stauffer 1993, Konings 1990), the populations of *Copadichromis* we sampled clearly form a distinct and monophyletic lineage. Konings (1990) suggested the placement of the volcano building and vertically barred *C. eucinostomus*, *C. prostoma*, and *C. boadzulu* within the genus *Nyassachromis*. While the bootstrap values strongly suggest that *C. borleyi* and *C. jacksoni* form a lineage that is sister to *C. eucinostomus* and *C. likomae*, we are unable to rule out an alternate topology where *C. eucinostomus* is basal to the other three species (KH p=0.111).

The four taxa of *Copadichromis* collected in this study make qualitatively different bowers. Two taxa display over bare rock: *Copadichromis borleyi* were observed defending territories over horizontal rock surfaces and *C. jacksoni* defended vertical rock surfaces.

128

Copadichromis likomae builds a large pit that may develop a short lip (Table 2). *Copadichromis eucinostomus* makes a symmetrical volcano bower in huge heterospecific leks (Table 3).

Protomelas

While Eccles and Trewavas (1989) erected the genus *Protomelas* without defining a shared synapomorphy, the taxa sampled in this study are strongly united. Within this clade, *P.* 'black rock' and *P. taeniolatus* cluster together to the exclusion of *P. similis* (KH p=0.017). All three taxa of this lineage share longitudinal dark bands. However Eccles and Trewavas did not consider this trait to be a synapomorphy and often gave more weight to trophic characters. The exclusion of the horizontally striped *Lethrinops lethrinus* and *L.* 'rock display' from this clade supports Eccles and Trewavas's (1989) assertion that this color pattern does not define a monophyletic group.

Protomelas taeniolatus defended territories over horizontal rock surfaces. Small aggregations of males (5-10 individuals) were seen displaying over small rings of sand and gravel placed on large rock boulders. These rings have the appearance of a squat volcano, but the platform surface is bare rock. *Protomelas similis* builds a pit bower with a sloped edge within beds of *Vallisneria* (Table 2). The undescribed species of *Protomelas* encountered at Black Rock makes a very tall and symmetrical volcano bower (Table 3). The sloping surface of these bowers were covered with hundreds of snail shells of the *Melanoides* genus (Fig. 2c). Interestingly, the pharyngeal jaws of P. 'Black Rock' have papilliform teeth, suggesting that this species does not prey upon snails.

While pharyngeal jaw dentition was similar for all *Protomelas* taxa, *P. taeniolatus* and *P.* 'Black Rock' possess different oral dentitions. As described by Eccles and Trewavas (1989), the

lower jaws of the observed *P. taeniolatus* have teeth arranged in a 4-5 series with the outer

bicuspid and inner tricuspid teeth. Conversely, the teeth of the lower jaw of *P.* 'Black Rock' are

in a 3-4 series with strongly recurved outer unicuspid teeth (some weakly bicuspid posterior) and

bicuspid teeth in the inner series.

Lethrinops

The genus *Lethrinops* (including *Tramitichromis* and *Taeniolethrinops*) is characterized

by a unique lower jaw dentition (Fig. 6) in which the outer tooth row curves inward to terminate

behind the inner row (Regan 1922). This distinguishes these genera from other haplochromines

in which the outer tooth row continues backward in a single series. Our phylogeny indicates that

the *Lethrinops* clade is paraphyletic: the genera *Copadichromis* and *Protomelas* are embedded

within this lineage. This would suggest that there has been parallel evolution of the

haplochromine-type dentition within Lake Malawi. Furthermore, the *Lethrinops* type dentition

does not define a monophyletic lineage.

The taxa *L. auritus*, *L. lethrinus*, and *L. parvidens* form a monophyletic lineage (KH

p=0.024). Within this clade, the mound building *L. auritus* and *L. lethrinus* do not cluster

together (KH p=0.037). As predicted by Eccles and Lewis (1979), and reaffirmed by Ngatunga

and Snoeks (2003), *L. auritus* and *L. parvidens* share a close affinity. The placement of *L.*

lethrinus within this clade is surprising since Eccles and Lewis (1978) suggested that *L.*

lethrinus, *L. argentea*, *L. leptodon*, and *L. lunaris* constitute a distinct lineage within *Lethrinops*.

As more *Lethrinops* spp. are added, the specific lineages with this old genus may be resolved.

Lethrinops lethrinus builds a pit bower with steep sides (Table 2), which is surrounded by

four or more ($\overline{X}$=8.1+/-SE=3.7) satellite mounds constructed of flocculent mud. A few *L.*

130

lethrinus individuals were observed with two pits within their territory. *Lethrinops auritus* builds a bower consisting of a large central mound (Fig. 2b) which is surrounded by 8-19 smaller satellite mounds ($\overline{X}$=12.9+/-SE=3.9) arranged in concentric circles. *Lethrinops parvidens* built the smallest volcano bower observed in this study (Table 3).

Tramitichromis

Eccles and Trewavas (1989) erected the new genus *Tramitichromis* to include species that had the *Lethrinops*-style dentition, but also have an anterior blade of the lower pharyngeal jaw that is directed downward instead of straight out (Fig. 6). The anterior blade of the pharyngeal jaw is the origin for the pharyngocleithralis externus muscle which inserts into the cleithrum, and the pharyngohyoideus muscle which inserts into the hyoid (Liem & Kaufman 1984). However this morphological character does not define a monophyletic lineage (KH p<0.0001). Both pharyngeal jaw dentition and gular colouration were highly variable within this lineage (Table 4). The position of *Tramitichromis variabilis* makes *Tramitichomis* paraphyletic as *Copadichromis*, *Protomelas*, and *Taeniolethrinops* are contained within this lineage.

One population within this lineage was found displaying over rock at Otter Point. These fish courted females from bare rock near the sand/rock interface. Like *Protomelas taeniolatus*, they moved small amounts of sand up to the rock and fashioned it in a ring. While we designated this population as *Lethrinops* 'rock display,' its taxonomic status is uncertain. As is typical for *Lethrinops*, the symphysis of the lower oral jaw expands to form a horizontal platform for the teeth (Trewavas 1931), yet the outer row of teeth extends back in a single series (haplochromine tooth form). While these fish have the 'haplochromine' oral dentition, they are strongly embedded within the *Tramitichromis* lineage (KH, p<0.001). While this finding is

131

initially surprising, *Lethrinops* 'rock display' is in the same clade as *Taeniolethrinops praeorbitalis*. Eccles and Trewavas (1989) have reported that specimens of another *Taeniolethrinops* species, *T. cyrtonotus*, also possess haplochromine-type oral dentition.

While all of the observed species of *Tramitichromis* built a volcano style bower, there was a great deal of quantitative variation (Table 3). Bowers built by *Tramitichromis* were significantly different for all measurements (ANOVA, $p<0.05$) except base diameter (ANOVA, $p=0.26$). This difference in bower size (bower volume) was not correlated with the mean standard length of the population (Pearson, $r=0.019$, $p=0.67$). With the exception of *T. variabilis*, all *Tramitichromis* bowers were constructed from fine sand with no perceptible difference in substrate consistency. Even though *T. variabilis* constructed a volcano composed of pea-sized gravel, the platform (often below the level of the substrate) was made of fine sand. Pairs of sister taxa within this lineage displayed repeated divergence of short-wide and tall-narrow volcano forms (Fig. 7).

Tramitichromis 'black rock' and *T.* 'microstoma-type' are reciprocally monophyletic sister taxa (KH $p=0.017$). *Tramitichromis* 'black rock' was confined to a small lek of five tall, narrow bowers (Table 3) at Black Rock Beach. These bowers abutted the shallow edge of a *Vallisneria* bed at 3m ($+/-SE=0$) of depth. Even though similar plant beds are located on Chembe Beach, no additional leks of *T.* 'black rock' were found. The population at Black Rock Beach was morphologically similar to *Tramitichromis* 'microstoma-type', which was collected on volcano bowers in 35ft of water off Otter Point. Unfortunately, time did not permit quantification of the bowers at Otter Point. In general, the *T.* 'black rock' bowers were taller and narrower than those of *T.* 'microstoma type'. Specimens from both populations possessed 100% papilliform pharyngeal dentition (Table 4).

132

<u>Tramitichromis liturus Species Group</u>

Members of the *Tramitichromis liturus* species group (McKaye *et al.* 1993, Stauffer *et al.*

2002) formed two pairs of parallel leks with distinct depth distributions (Fig. 1b). No

Tramitichromis or *Lethrinops* bowers were present in the intervening depths between these four

distinct leks. The two populations in each pair are reciprocally monophyletic sister taxa (KH

$p<0.01$).

In the first pair, individuals found in the shallow lek (2.0-3.0m $\overline{X}$=2.47m+/-SE=0.16)

constructed a highly symmetrical tall narrow bower with sharp crisp edges. The platform was

generally level and made of very finely packed sand (Fig. 8c). The lip of the volcano was

typically raised on one end to form a 'backsplash'. The platform area under this backsplash is

where egg deposition occurs and is made up of finely sifted sand. All males caught over these

tall, narrow bowers possessed a yellow gular (Fig. 8a). In contrast, all males caught in the

parallel, deeper lek (3.5-4.5m, $\overline{X}$=3.9m +/- SE=0.22) had pink-orange gulars (Fig. 8b).

Tramitichromis 'pink gular' constructed short, wide bowers with a curved or poorly defined

platform that occasionally sloped directly into the lake floor/substrate. The platform lip had

rounded edges and 2-3 raised sections (Fig. 8d). *Tramitichromis* 'pink gular' and *T.* 'yellow

gular' bowers were significantly different for every measurement (Table 3, ANOVA, $p <0.001$

for all measurements), except base diameter (ANOVA, $p=0.24$) and lip height (ANOVA,

$p=0.54$). Surprisingly, the slightly smaller (ANOVA, $p=0.058$) *T.* 'yellow gular' built the larger

bower. While superficially similar, *T.* 'pink gular' and *T.* 'yellow gular' possessed significantly

different pharyngeal dentition (Table 4, t-Test, $p<0.0001$). The lower pharyngeal jaw of *T.*

133

'yellow gular' had narrow posterior horns, and small papilliform teeth (Fig. 8e). Conversely, *T.* 'pink gular' had robust posterior horns and several large molariform teeth (Fig. 8f).

Approximately 200m east, a second pair of parallel *Tramitichromis* leks were found. These populations also appear to be reciprocally monophyletic sister taxa (KH p=0.013) and were designated *T.* 'deep' and *T.* 'shallow'. Males occupying the shallow lek (3.5-4.0m $\overline{X}$=3.69m+/-SE=0.16) constructed short, wide bowers with crisp lip edges and flat platforms (Table 3). Males in the deeper lek (4.5-5.5m $\overline{X}$=4.89m+/-SE=0.21) built tall, narrow bowers with rounded lip edges and slanted platforms, which were often oblong or triangular. *Tramitichromis* 'deep' and *T.* 'shallow' bowers were significantly different for every measurement (p<0.001 for all measurements), except base diameter (ANOVA, p=0.07) and lip height (ANOVA, p=0.81), but there was no discernable difference in each population's standard length (ANOVA, p=0.28). This pair of sister taxa also have similar pharyngeal dentition (Table 4, t-Test, p=0.39) and their gulars were a similar shade of yellow.

Speciation – Ecology vs. Sexual Selection

Our results show that both ecologically- and sexually-selected traits are differentiated among the most closely related species of bower building cichlids, lending credence to the idea that an interaction of natural and sexual selection is responsible for the rapid speciation of this group of fishes. The 3-stage model proposed by Kocher and Danley (2001) would suggest that sexual selection is the dominant force of divergence during recent speciation events. Every pair of sister taxa in this study differed in sexually selected characteristics and several sister taxa constructed significantly different bowers without apparent trophic differentiation. Species within the genus *Copadichromis* construct a wide range of bower types, but are all zooplanktivores (Turner 1996). The sister taxa *Tramitichromis* 'deep' and *T.* 'shallow' share similar oral and pharyngeal dentitions and have leks that are separated by only 1m of depth, yet they build bowers that are significantly different in size. Similarly, both *T.* 'black rock' and *T.* 'microstoma type' share similar trophic morphology, but build their bowers in different habitats. These taxa are congruent with models that propose that sexual selection can drive speciation without ecological differentiation (Wu 1985, Turner & Burrows 1995, Higashi *et al.* 1999, Takimoto *et al.* 2000, Lande *et al.* 2001). Intrageneric comparisons of other rift valley cichlid species have also generated empirical support for these models. Mate choice experiments have demonstrated that colour morphs of the Lake Victorian cichlid, *Haplochromis nyererei*, are reproductively isolated even though they are ecologically indistinguishable in the wild (Seehausen & van Alphen 1998). Similarly, species within the Lake Malawi genus *Metriaclima*

display a dramatic diversity of male colour pattern yet show little ecological divergence (Danley & Kocher 2001).

While all pairs of sister taxa constructed different bowers, several taxa were also trophically differentiated. *Tramitichromis* 'pink gular' and *T.* 'yellow gular' possess different pharyngeal jaw morphologies, in addition to differences in gular colour and bower form (Fig. 8). The robust muscular process and large molariform teeth of *T.* 'pink gular' is indicative of a diet based on gastropods or shelled insects (Barel *et al.* 1977, Greenwood 1984, Trapani 2003). In contrast the slender muscular process and narrow papilliform teeth of *T.* 'yellow gular' are associated with a diet of softer benthic invertebrates. Louda *et al.* (1983) found that maximum gastropod densities occur over a very narrow depth range (1.5-4.5m), suggesting that competition for trophic resources may exist over restricted spatial scales. Within this same lineage, *Lethrinops* 'rock display' also possess molariform pharyngeal teeth while *Taeniolethrinops praeorbitalis* has papilliform dentition. While more populations need to be sampled to ascertain the relationship between these two taxa, it is clear that there has been repeated trophic divergence within this lineage (Fig. 7). Additionally, ecological divergence (inferred from morphology) has been reported for species within the sand dwelling genera *Trematocranus* (Eccles & Trewavas 1989), *Otopharynx* (Arnegard & Snoeks 2001) and *Protomelas* (Stauffer 1993).

While this pattern of differentiation may be specific to the sand dwelling lineage, ecological divergence appears to be common in many other cichlid lineages. Trophic differentiation has been reported for species within Lake Malawi's rock dwelling genus *Melanochromis* (Bower & Stauffer 1997) and subgenus *Pseudotropheus tropheops* (Genner *et al.* 1999, Albertson 2002). The oral dentition of the Eretmodini tribe in Lake Tanganyika exhibits

136

considerable morphological differentiation (Poll 1986). Species of Lake Natron and Lake Magadi tilapiine flocks have divergent oral dentition (Tichy & Seegers 1999). Trophic specialization has also been important during the formation of the West African (Schliewen *et al.* 2001) and Neotropical (Barluenga & Meyer 2004) crater-lake species flocks.

The divergence of sister taxa by both natural and sexual selection is not uncommon among cichlid lineages. In addition to those taxa observed in this study, many closely related cichlid species differ in pharyngeal jaw morphology (Eccles & Trewavas 1989, Smits *et al.* 1996, McKaye *et al.* 2002, Barluenga & Meyer 2004). Unlike the morphological characters important during stickleback (Rundle *et al.* 2000) or *Anolis* trophic differentiation, it is difficult to envision direct female choice for divergent pharyngeal jaw dentition. In Darwin's finches male courtship song is strongly influenced by beak morphology (Podos 2001). Similarly, several sand dwelling cichlids also make sound during courtship (Lobel 1998). However, while stridulation of the pharyngeal apparatus produces these sounds, they appear to be genus-specific rather than species-specific. Although the pharyngeal jaw activity of *Tramitichromis intermedius* appears to be sexually dimorphic (Rice & Lobel 2002), there is not yet evidence of female preference for any sound characteristic. Additionally, there is no obvious direct connection between pharyngeal jaw morphology and either gular colour or bower form. While the genetic architecture underlying these traits is currently unknown, quantitative trait loci (QTL) studies may give us a better understanding of how each character is shaped by both natural and sexual selection (Albertson *et al.* 2003).

Meyer (1987) suggested that phenotypic plasticity could explain the majority of morphological variation observed within the African species flocks. However, this was predicated on the assumption that New and Old World cichlids displayed a similar magnitude of

137

plasticity. Meyer further noted that the results of Witte (1984) suggested that the effect of plasticity was less pronounced in Old World lineages, a result further corroborated by Smits *et al.* (1996) and Huysseune (1995). While both studies demonstrated that diet induced morphological plasticity of the lower pharyngeal jaw, this variation is not sufficient to fully explain the magnitude of interspecific variation observed in this study. Further there are reports of strong genic control of pharyngeal jaw dentition in both New (Trapani 2003) and Old World (Smits *et al.* 1996) lineages. Kornfield and Taylor (1983) suggest that pharyngeal dentition (molariform vs. papilliform) is controlled by a single locus, with papilliform dominant to the molariform dentition. Even if the morphological variation observed in this study is the result of phenotypic plasticity, it would still indicate that some sister taxa are trophically divergent regardless of the mechanism of morphological differentiation (Trapani 2003).

Bower Evolution

Bower form is more evolutionarily labile than previously expected. McKaye (1991) was the first to suggest that species with similar bower forms have arisen from a common ancestor. Stauffer (2003) further suggested that general bower design could be used as a genus defining synapomorphy, implying phylogenetic constraint. However our AFLP phylogeny indicates that multiple bower forms are found within the same lineage. Furthermore, both the pit and volcano bower forms are found in each major lineage, indicating repeated convergence on these forms. While mound building is found within only one major lineage, it does not appear to define a monophyletic group of species. These results are in sharp contrast to bowerbirds in which the two major bower forms (avenue and maypole) form distinct clades (Kusmierski *et al.* 1993, Kusmierski *et al.* 1997).

138

While bower form is not a reliable character for defining genera, it does appear to be species-specific. AFLP markers clearly demonstrate that populations which differ qualitatively or quantitatively in bower forms are genetically distinct species. Each population that we initially identified by bower type, even those separated by only a meter in depth, appears to be a genetically distinct species. For example, *Lethrinops* populations from opposite sides of the Nankumba peninsula, but which make the same bower, are more closely related to each other than are *Tramitichromis* populations that make slightly different bowers only a meter apart. This result tends to support the use of bower shape in the definition of species (e.g. Stauffer *et al.* 1993).

Even though bower form appears to be species specific, we must be cautious using bower form as a taxonomic character due to our lack of knowledge concerning the relative importance of genetics and environment on bower form. Certainly, the bowers of some sister taxa differ by discrete changes. The *Copadichromis* lineage appears to be made of two distinct clades: rock and sand based bower types. *Protomelas taeniolatus* carry sand up to the rock surface to form rings that are reminiscent of shallow volcanoes and are more closely related to the volcano building species than they are to the pit building *P. similis*. Many sister taxa within the *Tramitichromis* lineage show replicated divergence (Fig. 7) of volcano size (tall narrow/short wide), location (depth, weed bed), and lip/crown construction (defined/undefined). However, while McKaye *et al.* (1993) suggested that divergence in bower form was correlated with genetic distance, there is no direct evidence that bower form is heritable. Only one species in this study was sampled at multiple locations (*Lethrinops auritus*), so the magnitude of intraspecific bower variation is unknown. Stauffer and Sato (2002) suggest that the newly described *Copadichromis atripinnis* may be closely related to *C. geertsi*, and *C. trewavasae* due to the shared incorporation

139

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of a rock in their bower. However Lake Tanganyika's *Cyathopharynx furcifer* exhibits geographic variation in rock use during bower construction (Rossiter & Yamagishi 1997). Further geographic sampling and rigorous laboratory experimentation will be necessary to tease apart the relative effects of genetics and environment on bower construction.

While the interplay of forces shaping cichlid bowers are still poorly understood, it has been suggested that bower evolution is driven by intense sexual selection via female choice (McKaye *et al.* 1990, McKaye *et al.* 1993, Stauffer & Kellogg 1996). McKaye (1991) has suggested that the wide range of bower forms utilized by Lake Malawi cichlids implies that there is no adaptive 'optimum' bower form. The great variation we observe in bower form may be driven entirely by sexual selection. Even though males provide no resources or parental care, many studies have demonstrated female mating preferences within the lek (McKaye *et al.* 1990, McKaye 1991, Stauffer & Kellogg 1996, Kellogg *et al.* 2000). Female *Copadichromis eucinostomus* preferentially visited and circled with males over taller bowers (McKaye *et al.* 1990). Similarly, Kellogg *et al.* (2000) were able to correlate bower height and location with female visitation in *Lethrinops parvidens*. However, they were unable to find any bower characteristic that was correlated to the later stages of the spawning sequence: circling and spawning. Female *Otopharynx argyrosoma* choose centrally located bowers, regardless of height (McKaye 1991). However, it is still uncertain if aspects of bower shape or location provide a direct selective advantage to the parents through increased protection against egg predation by hetero- and conspecifics (McKaye 1984).

Most discussions of bower evolution have bypassed the questions regarding the role of natural selection and bower heritability, and instead have focused on which model of sexual selection is appropriate. McKaye (1991) proposed that bower divergence is driven by Fisherian

runaway selection as modeled by Lande (1981, 1982). While variation in the male character is well documented (McKaye 1984, McKaye et al. 1990, McKaye 1991, Kellogg et al. 2000) variation in female preference is assumed from variable male success (McKaye 1991). However, this ignores the possibility that females utilize multiple cues during male assessment. Additionally, no data concerning the heritability of either bower form or female preference have ever been presented. Taylor et al. (1998) found a negative correlation between parasite load and male reproductive success, as well as a positive correlation between parasite load and bower height skew (ratio of minimum and maximum bower height) in C. eucinostomus. While this suggests that bower shape may be an honest indicator of a male parasite resistance, Taylor et al. (1998) did not find any correlation between bower height skew and female preference. Seehausen and Schluter (2004) have recently suggested that character displacement due to male-male aggression has driven the diversification of Lake Victoria's rock dwelling cichlids. Males possessing a novel colour pattern would receive less aggression from nearby conspecifics and have a selective advantage during territory acquisition. If applied to Lake Malawi's bower building cichlids, this model would predict that bowers of recently evolved sister taxa would be interdigitated on the same area. Our results indicated that while leks of different species often overlap, recently evolved sister taxa are found in mutually exclusive leks (Fig. 1b). Undoubtedly, individual bower characters have different or multiple discrete functions (conspecific recognition, male/male competition, intraspecific female choice, ecological constraint, reduction of egg predation). Rigorous experimental manipulation will be needed to tease apart the various aspects of individual character function.

Sand Dweller Evolution and Diversity

This study has identified several cryptic species within the genus *Tramitichromis*, suggesting that sand dweller species richness has been substantially underestimated. Genner *et al.* (2004) contend that estimates of Lake Malawi species richness have been exaggerated due to the incorporation of putative allopatric species. They achieved a 'more realistic' estimate of species richness by collapsing these estimates and limiting alpha taxa to species, or colour variants, that can be found in sympatry. However the presence of cryptic species makes estimating alpha diversity very difficult. McKaye *et al.* (1993) recognized 5 distinct taxa within what they referred to as the *Tramitichromis liturus* species group, however they were unable to differentiate between populations using allozyme markers. Turner (1996) agreed that *Tramitichromis liturus* was poorly defined and was likely a complex of related species. On Chembe Beach we have identified 7 separate species belonging to the genus *Tramitichromis*. Of the 5 species currently recognized (Eccles & Trewavas 1989), only 2 descriptions correspond with the species we surveyed at Chembe Beach. Continued sampling of additional beaches and taxa may uncover more cryptic species and result in a startling increase in the magnitude of species richness in Lake Malawi cichlids.

Populations of sand dwellers demonstrate a higher degree of population substructuring than previously expected. In contrast to the stenotypic mbuna, species within the sand dwelling lineage were thought have large effective population sizes and wider geographical distributions (Turner 2000, Taylor & Verheyen 2001). This impression has been bolstered by recent studies that found no significant population substructuring in pelagic *Diplotaxon* species (Shaw *et al.* 2000) or populations of the sand dwelling *P. similis* (Pereyra *et al.* 2004). However, Taylor and Verheyen (2001) were able to detect weak, but significant structure between widely dispersed

142

Copadichromis 'virginalis kajose' and the lithophilic *Protomelas taeniolatus*. Our AFLP data clearly resolved the populations of *Lethrinops auritus* collected from opposite sides of the Nankumba Peninsula. Additional collections will be needed to assess the effect of migration on the evolution of these diverse sand dwelling communities and its potential impact on fisheries conservation.

The stunning diversity of Lake Malawi cichlids will require rigorous molecular studies to identify distinct species and identify appropriate units of conservation (Soltis *et al.* 1999). Sand dwelling cichlids make up a vital component of the Malawian diet (Munthali 1997, Ribbink *et al.* 2001) and are heavily fished. *Copadichromis* and *Lethrinops* species are a significant portion of both artisanal and commercial harvests (Turner 1994, Duponchelle & Ribbink 2000). However, despite their economic importance, our knowledge of sand dweller taxonomy is extremely limited. At least 30-50% of the species lack formal description (Turner 1996) and many of the current species descriptions are based on a single museum specimen or lack a type locality (Eccles & Trewavas 1989). Systematic collections of sand dwelling cichlids were traditionally performed using demersal trawling, which would fail to perceive the cryptic diversity identified in this study. Barlow (2002) has indicated that the technology is available for long-term storage of behavioural data. Coupled with improving database technology for molecular data (Hong & Chuah 2003), the holistic holotype necessary to describe species in this incredibly diverse system is within our grasp.

Conclusions

The ever-increasing complexity of the Lake Malawi system continues to challenge our ability to model the forces behind cichlid diversification. Our results suggest that the forces of

143

natural and sexual selection work both independently and synergistically during the formation of new species within this natural laboratory of evolution. The parallel evolution of colour pattern, trophic morphology and bower form diminish their effectiveness as taxonomic characters. The co-occurrence of sister taxa separated by only 1-2 meters of depth suggests strong isolating mechanisms and repeated displacement of depth preference during sympatric speciation or colonization by allopatric taxa during lake level fluctuations. The presence of cryptic species suggests a severe underestimation of the species diversity within the sand dwelling cichlid lineage and will complicate fisheries and conservation policy.

Acknowledgments

Table 5.1

Taxa of bower building cichlids sampled for AFLP fingerprint analysis.

Genus	Species	Collection Site	Bower Type
Copadichromis	*eucinostomus*	Chembe Beach	Volcano
Copadichromis	*likomae*	Otter Point	Pit
Copadichromis	*jacksoni*	Otter Point	Vertical Rock Surface
Copadichromis	*borleyi*	Otter Point	Horizontal Rock Surface
Protomelas	*similis*	Otter Point	Pit
Protomelas	*taeniolatus*	Otter Point	Horizontal Rock Surface
Protomelas	'black rock'	Black Rock Beach	Volcano
Tramitichromis	*variabilis*	Otter Point	Pit-Volcano
Tramitichromis	'microstoma-type'	Otter Point	Volcano
Tramitichromis	'black rock'	Black Rock Beach	Volcano
Tramitichromis	'deep'	Chembe Beach	Volcano
Tramitichromis	'shallow'	Chembe Beach	Volcano
Tramitichromis	'yellow gular'	Chembe Beach	Volcano
Tramitichromis	'pink gular'	Chembe Beach	Volcano
Lethrinops	*auritus*	Chembe Beach	Mounds
Lethrinops	*auritus*	Songwe Hill	Mounds
Lethrinops	rock display	Otter Point	Horizontal Rock Surface
Lethrinops	*lethrinus*	Chembe Beach	Pit with Mounds
Lethrinops	*parvidens*	Songwe Hill	Volcano
Taeniolethrinops	*praeorbitalis*	Chembe Beach	Pit
Outgroups:			
Metriaclima	*zebra*	Mumbo Island	Cave
Metriaclima	*zebra*	Domwe Island	Cave
Rhamphichromis	sp.	Chembe Beach	None Reported
Tropheus	*duboisi*	Lake Tanganyika	Horizontal Rock Surface

145

1 **Table 5.2**

2 Means and standard deviations recorded for all measurements of pit-type bower dimensions. The pit shape was significantly different

3 (ANOVA, p<0.001) for all measurements.

4

5

Species (n)	Pit Depth (mm)	Pit Diameter (mm)		# Satellite	Water Depth
	Max. (std)	Max. (std)	Min. (std)	Mounds (std)	m (std)
Copadichromis likomae (6)	228.0 (77.9)	742.0 (170.4)	742.0 (170)	0	NR
Protomelas similis (7)	80.0 (28.9)	317.1 (68.2)	276.0 (38.2)	0	NR ~1.2
Taeniolethrinops praeorbitalis (7)	442.5 (220.3)	1583.0 (167)	NR	0	5.1 (0.7)
Lethrinops lethrinus (7)	116.7 (16.3)	NR	NR	8.1 (3.7)	4.1 (0.16)

146

Table 5.3

Means and standard deviations of measurements taken from ten populations that build volcano-type bowers. Bower dimensions differed significantly (ANOVA, $p < 0.001$ for all measurements) between taxa.

Species (n)	Total Height (mm)		Lip Height (mm)		Base Diameter (mm)		Platform Diameter (mm)		Slope (mm)	Water Depth
	Max. (std)	Min. (std)	Max. (std)	Min. (std)	Max. (std)	Min. (std)	Max. (std)	Min. (std)	Max. (std)	(m) (std)
Copadichromis eucinostomus (6)	83.3 (21.6)	83.0(22.0)	53.3 (13.6)	53.3 (13.6)	475.0 (88.9)	475.0 (88.9)	230.0 (30.3)	230.0 (30.3)	NR	NR
Protomelas sp. 'black rock' (6)	271.7 (43.1)	271.7 (43.1)	49.2 (10.2)	45.0 (17.3)	1253.3 (131.6)	1253.3 (131.6)	481.7 (47.9)	481.7 (47.9)	445.0 (97.5)	6.8 (0.36)
Tramitichromis 'yellow gular' (11)	253.6 (67.3)	211.0 (93)	61.8 (22.7)	17.4 (10.7)	1263.6 (253.7)	1263.6 (253.7)	412.3 (56.5)	412.3 (56.5)	478.2 (156.0)	2.5 (0.17)
Tramitichromis 'pink gular' (7)	159.2 (47.2)	123.0 (48)	68.3 (15.7)	40.0 (13)	1096.7 (305.7)	1018.0 (237)	515.0 (87.3)	485.0 (74)	259.0 (79)	3.9 (0.23)
Tramitichromis 'deep' (10)	291.0 (80.8)	291.0 (80.8)	68.0 (25.6)	22.0 (7.9)	1379.0 (276.5)	1262.0 (247)	456.0 (105.5)	375.0 (47.4)	470.0 (118.1)	4.9 (0.21)
Tramitichromis 'shallow' (7)	182.9 (54.7)	182.9 (54.7)	65.0 (24.7)	57.9 (23.4)	1172.9 (74.1)	1133.0 (81.8)	540.0 (60.8)	491.0 (108)	310.0 (46.2)	3.7 (0.12)
Tramitichromis 'microstoma-type' (0)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR ~11
Tramitichromis 'black rock' (5)	310.0 (59.6)	290.0 (39)	32.0 (13.5)	30.0 (11.7)	1182.0 (174.6)	1182.0 (174.6)	364.0 (39.1)	364.0 (39.1)	498.0 (102.8)	2.7 (0.0)
Tramitichromis variables (8)	174.4 (51.6)	161.0 (66)	96.3 (43.7)	79.4 (23.1)	1036.9 (158.9)	1036.9 (158.9)	698.1 (113.3)	698.1 (113.3)	233.3 (110.15)	6.8 (2.41)
Lethrinops parvidens (7)	62.9 (20.6)	61.0 (19)	52.1 (12.9)	40.0 (5.8)	474.3 (91.3)	474.3 (91.3)	317.1 (52.5)	317.1 (52.5)	94.3 (29.9)	4.9 (0.12)

147

Table 5.4

Mean proportion of molariform teeth and gular color for all *Tramitichromis* taxa sampled.

Tramitichromis spp.	Mean Proportion Molariform Teeth	Gular Color (pink/yellow)
T. 'pink gular'	0.21	pink
T. 'yellow gular'	0.00	yellow
T. 'deep'	0.17	yellow
T. 'shallow'	0.18	yellow
T. 'black rock'	0.00	yellow
T. 'microstoma-type'	0.00	yellow
T. variabilis	0.00	polymorphic

148

Figure 5.1

Sample locations along the Nankumba Peninsula in southern Lake Malawi. (a) Bower building taxa were collected from Black Rock Beach, Otter Point, Chembe Beach, and Songwe Hill. (b) Enlarged section of Chembe Beach displaying the distribution of *Tramitichromis* bowers. Sister taxa occupied parallel leks with a very narrow depth distribution (separated by 1-1.5m of depth). While these leks overlapped with *Copadichromis eucinostomus*, they did not overlap with other *Tramitichromis* or *Lethrinops* species. No *Tramitichromis* or *Lethrinops* bowers were found in the intervening areas.
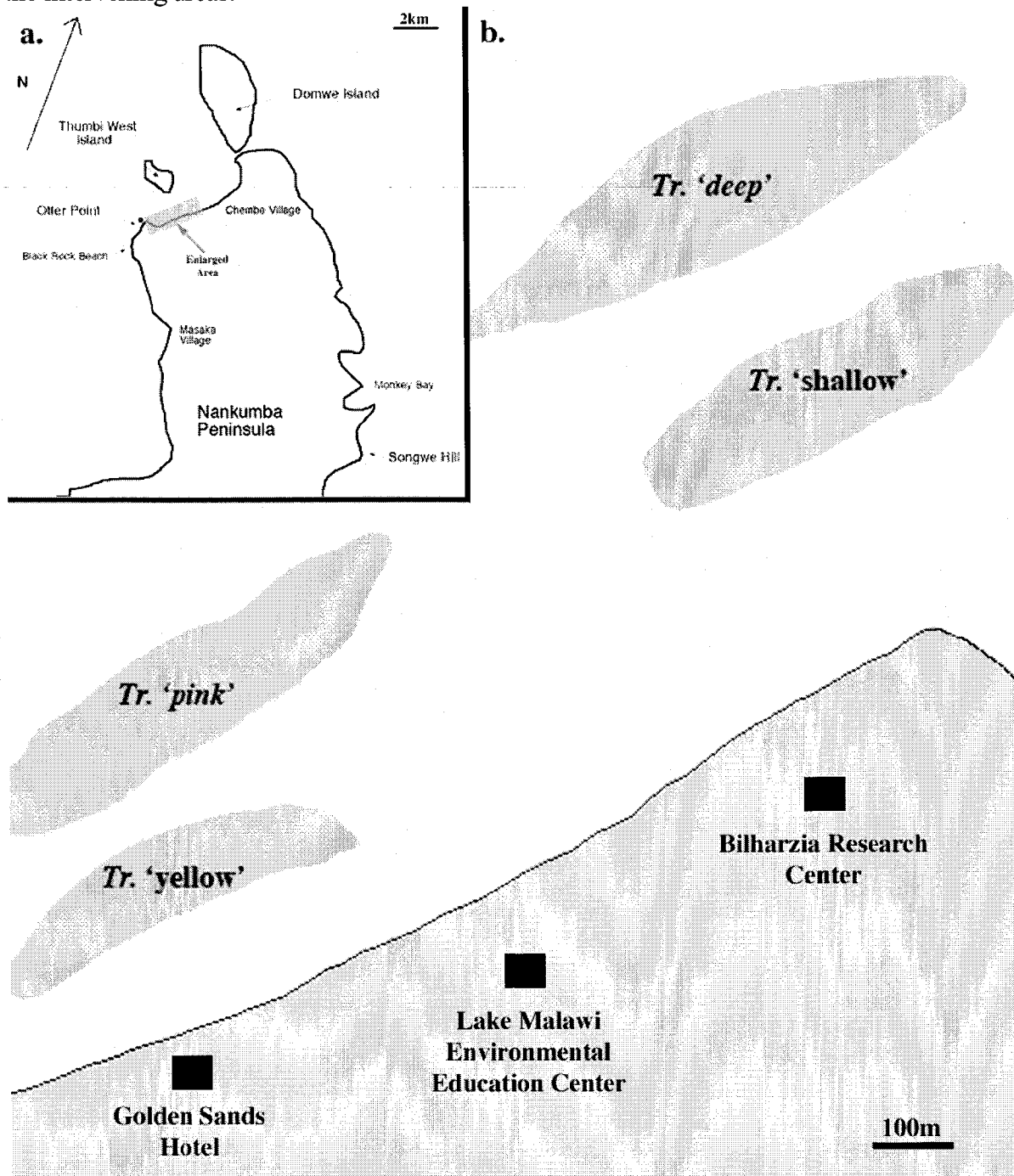

149

Figure 5.2

Basic bower forms built by the cichlid fishes of Lake Malawi. (a) Typical pit bower constructed by *Dimidiochromis kiwinge*. (b) Display sites built by *Lethrinops auritus* consist of a central mound surrounded by smaller satellite mounds. (c) *Protomelas* 'Black Rock' builds a typical volcano (or cone) type bower. Egg deposition occurs in a cup-like depression against the lip of the raised platform. All of the bowers built by this population were covered with snail shells (*Melanoides sp.*). (d) *Tramitichromis* sp. 'yellow throat' also builds a volcano style bower. Several *Tramitichromis spp.* build one or more raised sections on the platform lip (backsplashes) typically near the site of egg deposition. (e) Sketch of a volcano bower indicating the measurements used to identify populations that differed in bower form (after Kellogg *et al.* 2000). Minimum and maximum values of each measurement were used to quantify bower shape.

150
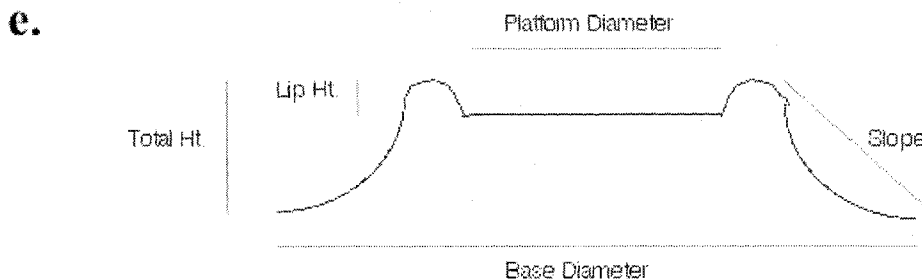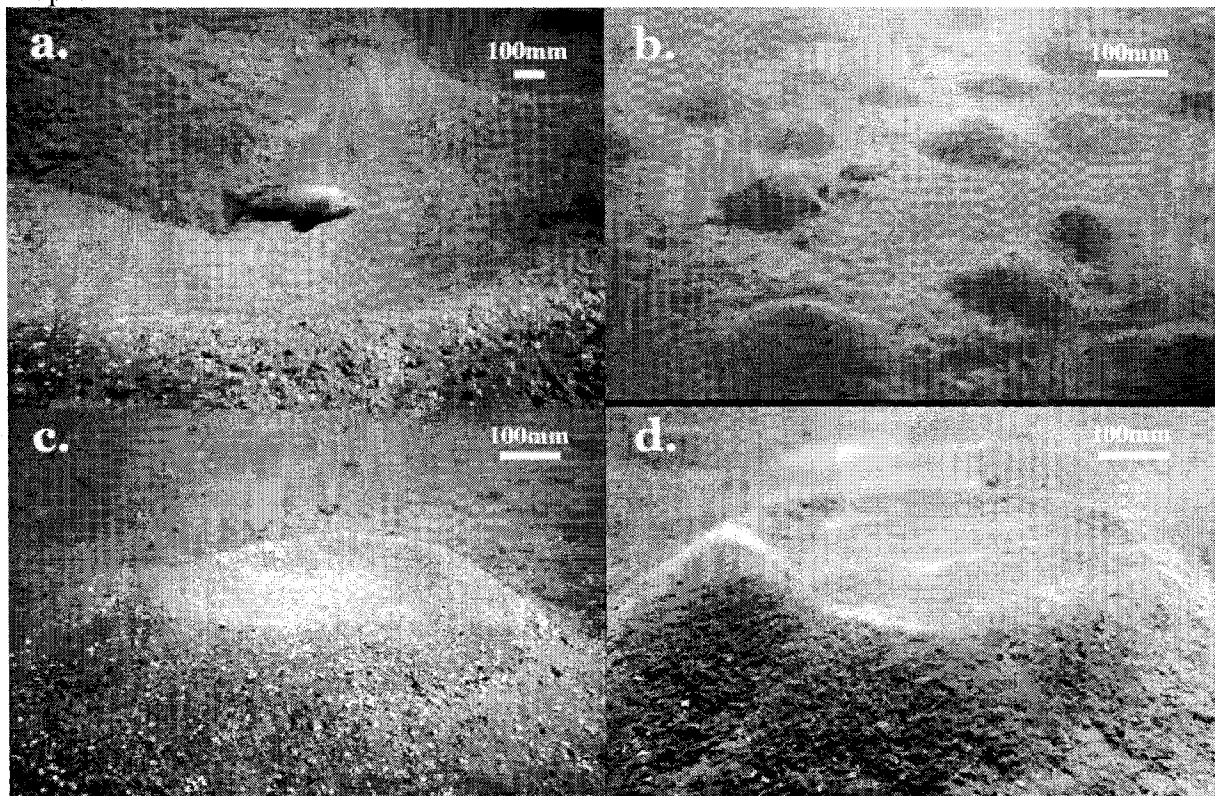
Figure 5.3
Dendrogram of the bower building cichlids of Lake Malawi based on Nei Li's genetic distance
and constructed using the Neighbor Joining algorithm.

151
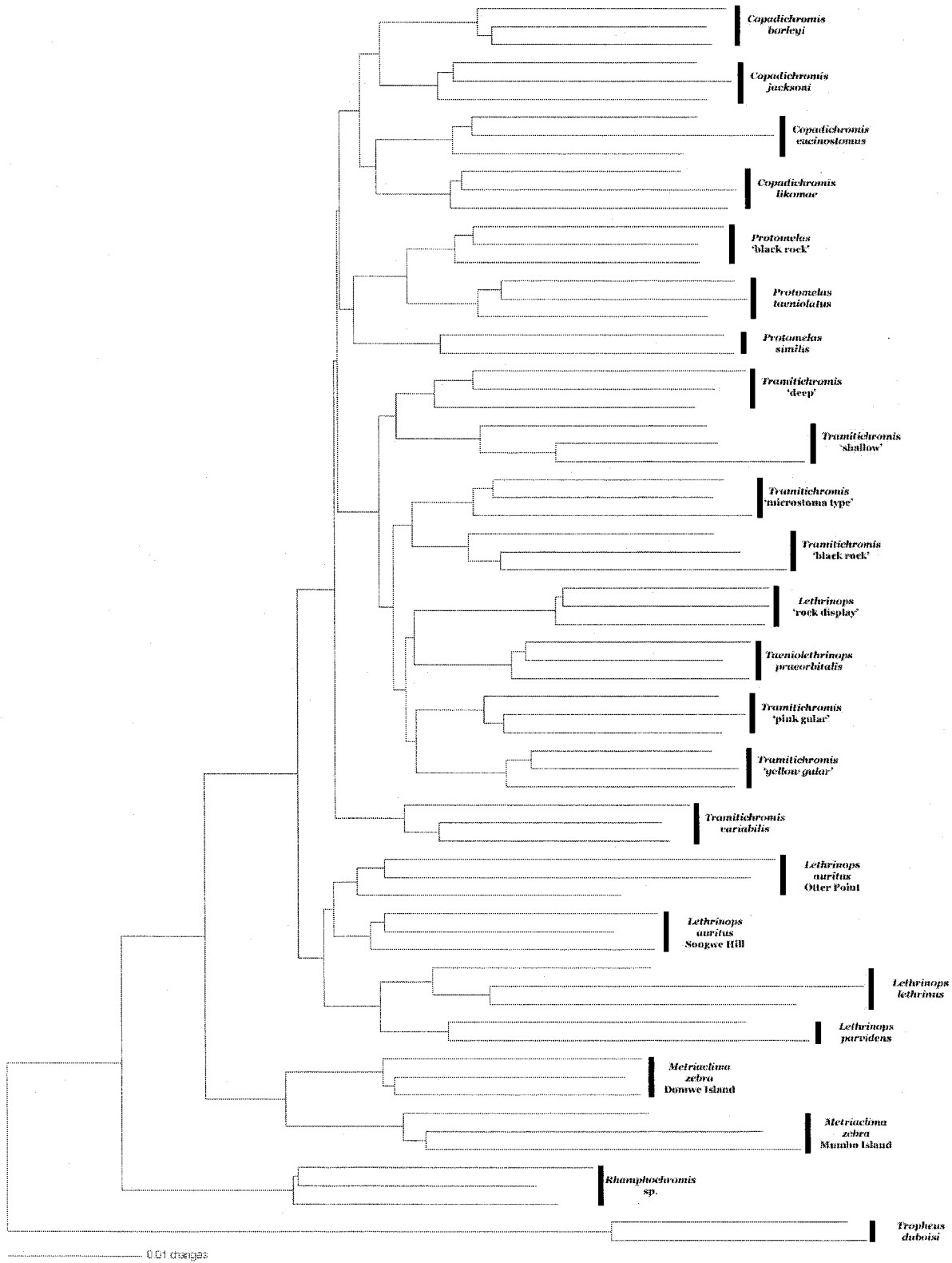
Figure 5.4

Phylogeny of bower building cichlids of Lake Malawi based on Nei Li's genetic distance and constructed using the Neighbor Joining algorithm. Numbers at each node indicate the bootstrap values (from 1,000 replicates) for that node. All but one node is resolved above a 50% bootstrap value. Symbols indicate the general bower type (pit, volcano, rock and mound) constructed by each population. The cichlid diagrams illustrate the melanin color pattern of each population. Bars on the right indicate the current generic assignment of each taxon.

152
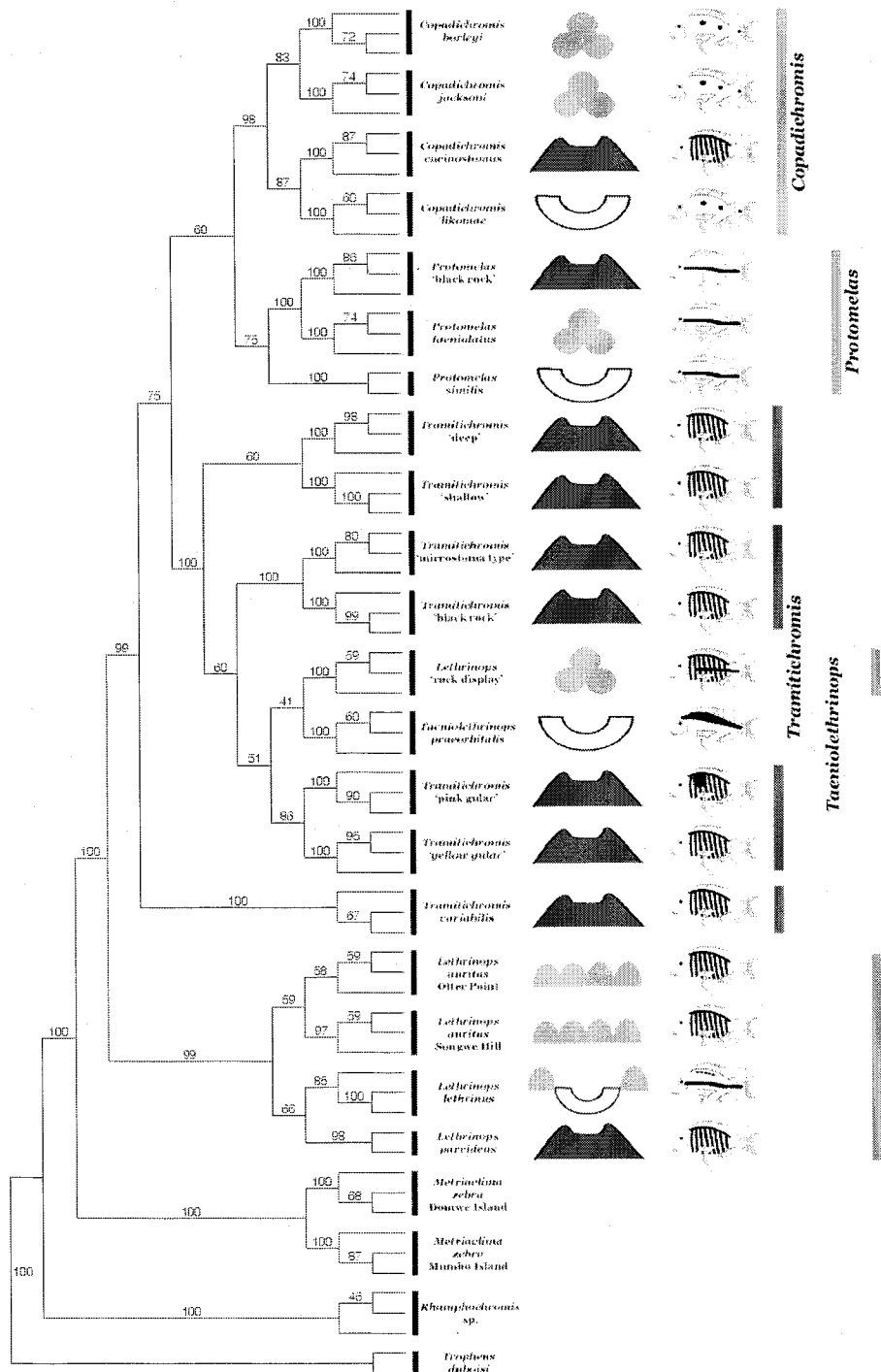
Figure 5.5

The box plot of the distribution of bootstrap support values (%) for the node uniting *Lethrinops lethrinus* and *L. parvidens* during the sequential removal of each taxa (a). Only the removal of both populations of *L. auritus* (open circle) resulted in a substantial increase in bootstrap support (100%). Additionally the graphical representation (splits graph) of the split decomposition of the distance matrix (b) suggests that instead of a bifurcating tree, a network better explains the relationship between *L. auritus*, *L. lethrinus*, and *L. parvidens*.

a.

60 70 80 90 100 110

b.

L. parvidens
Songwe Hill

L. parvidens
Songwe Hill

Rhamphachromis
sp.

L. aurita
Songwe Hill

Rhamphachromis
sp.

Rhamphachromis
sp.

L. lethrinus
Chembe Beach

L. lethrinus
Chembe Beach

L. lethrinus
Chembe Beach

Lethrinops aurita
Chembe Beach

Lethrinops aurita
Chembe Beach

L. aurita
Songwe Hill

L. aurita
Songwe Hill

Lethrinops aurita
Chembe Beach

0.01

Fit=78.3 ntax=14

153

Figure 5.6

Pharyngeal and oral jaw morphology used to classify Lake Malawi cichlid taxa. The upper pair of photos displays a lateral view of the lower pharyngeal jaw of (a) *Taeniolethrinops praeorbitalis* and (b) *Tramitichromis variabilis*. The ventral inclination of the anterior blade distinguishes members of the genus *Tramitichromis* from *Lethrinops* and *Taeniolethrinops*. The lower pair of photos displays a dorsal view of the lower oral jaws of (c) *Protomelas similis* and (d) *Taeniolethrinops praeorbitalis*. Arrows to the left and right of the jaws indicate the termination of the inner and outer (respectively) rows of teeth. Typical 'haplochromine' dentition (c) is characterized by the outer row of oral dentition continuing posteriorly after the termination of the inner row. In contrast, *Lethrinops* dentition (d) is defined by the outer row of teeth curving inward and terminating immediately behind the inner row of teeth.

154
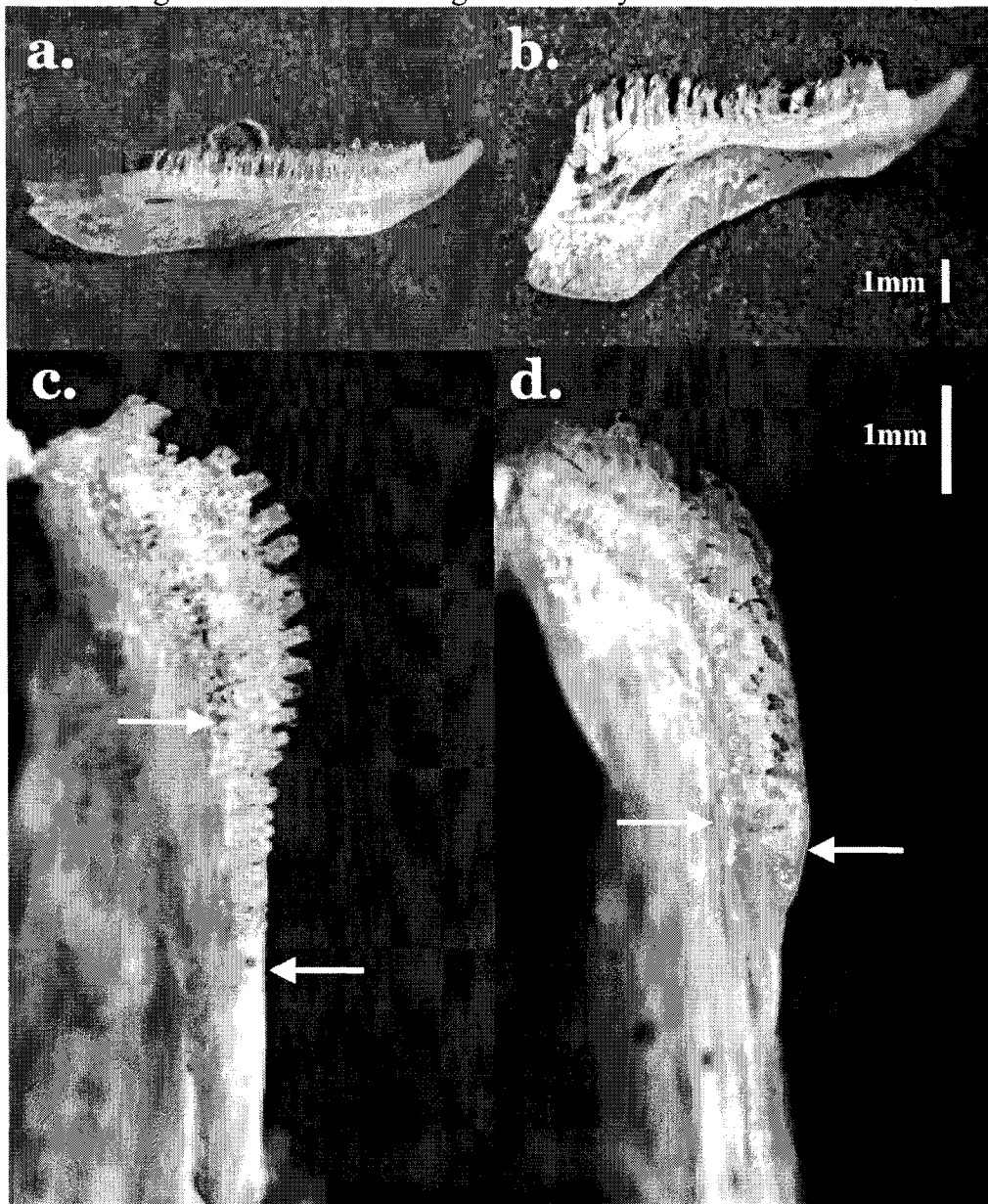
Figure 5.7

The *Tramitichromis* lineage demonstrates parallel evolution of bower form and pharyngeal jaw dentition. While each pair of sister taxa show replicated evolution of tall/narrow vs. short/wide volcano forms, these species exhibit different degrees of trophic differentiation. *Tramitichromis* 'deep' and *T.* 'shallow' possess lower pharyngeal jaws which share a similar molariform dentition. The pharyngeal jaws of both *T.* 'black rock' and *T.* 'microstoma type' have papilliform dentition. In contrast, *T.* 'yellow gular' and *T.* 'pink gular' have divergent pharyngeal tooth morphology.

155
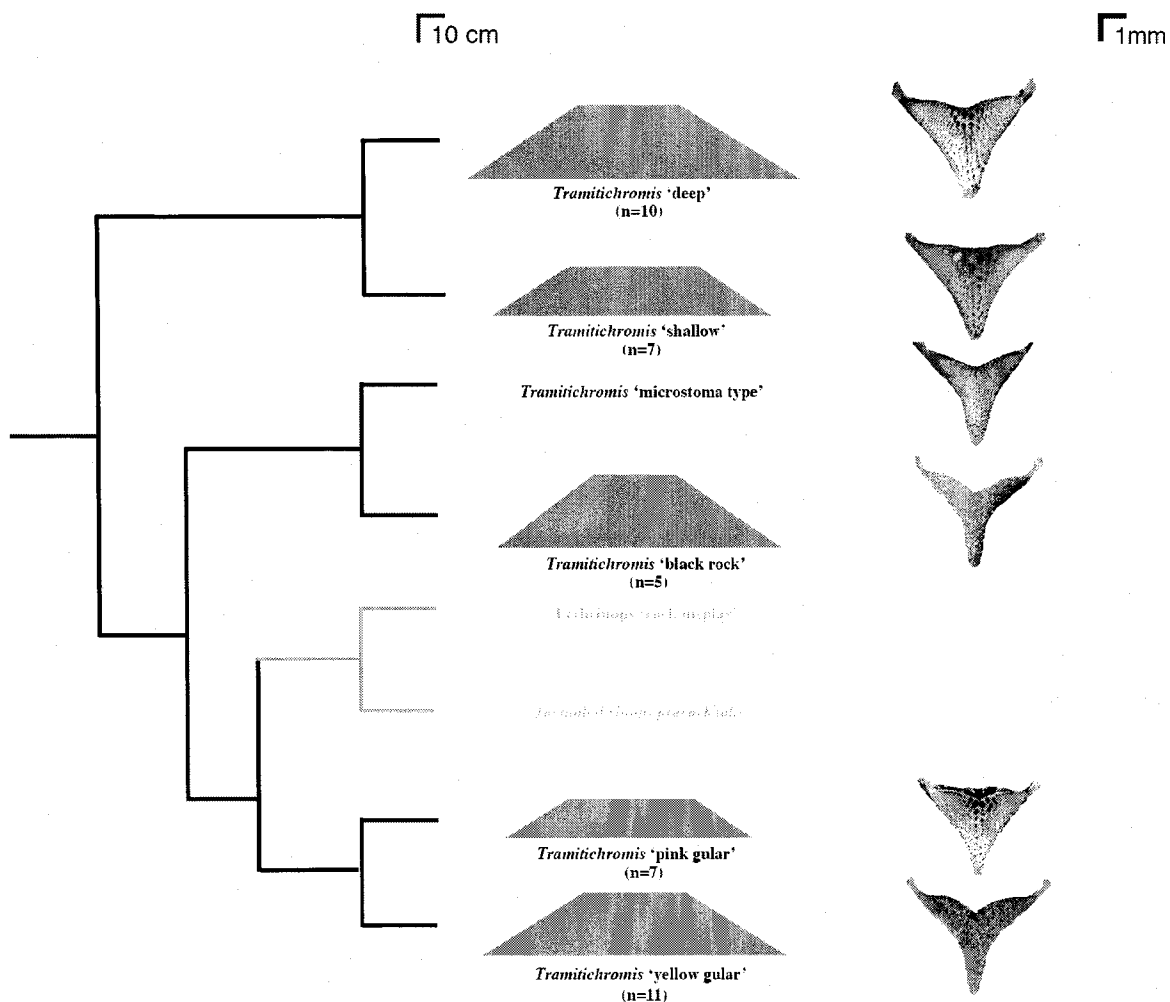
Figure 5.8

Morphological and behavioral differentiation of *Tramitichromis liturus* 'yellow gular' (a) and *T. liturus* 'pink gular' (b). Despite being separated by less than 1.5m in depth, these sister taxa are clearly divergent in gular color, bower form and trophic morphology. *Tramitichromis* 'yellow gular' (c) constructs a tall narrow bower with a level platform and one backsplash (raised platform lip). In contrast, *T.* 'pink gular' (d) constructs a shorter and wider bower (ANOVA, p<0.001) with a concave or slanted platform and 2-3 backsplashes. The lower pharyngeal jaw of *T.* 'yellow gular' has fine papilliform teeth (e), while *T.* 'pink gular' has a larger proportion of molariform teeth (f)(t-Test, p<0.0001).

156
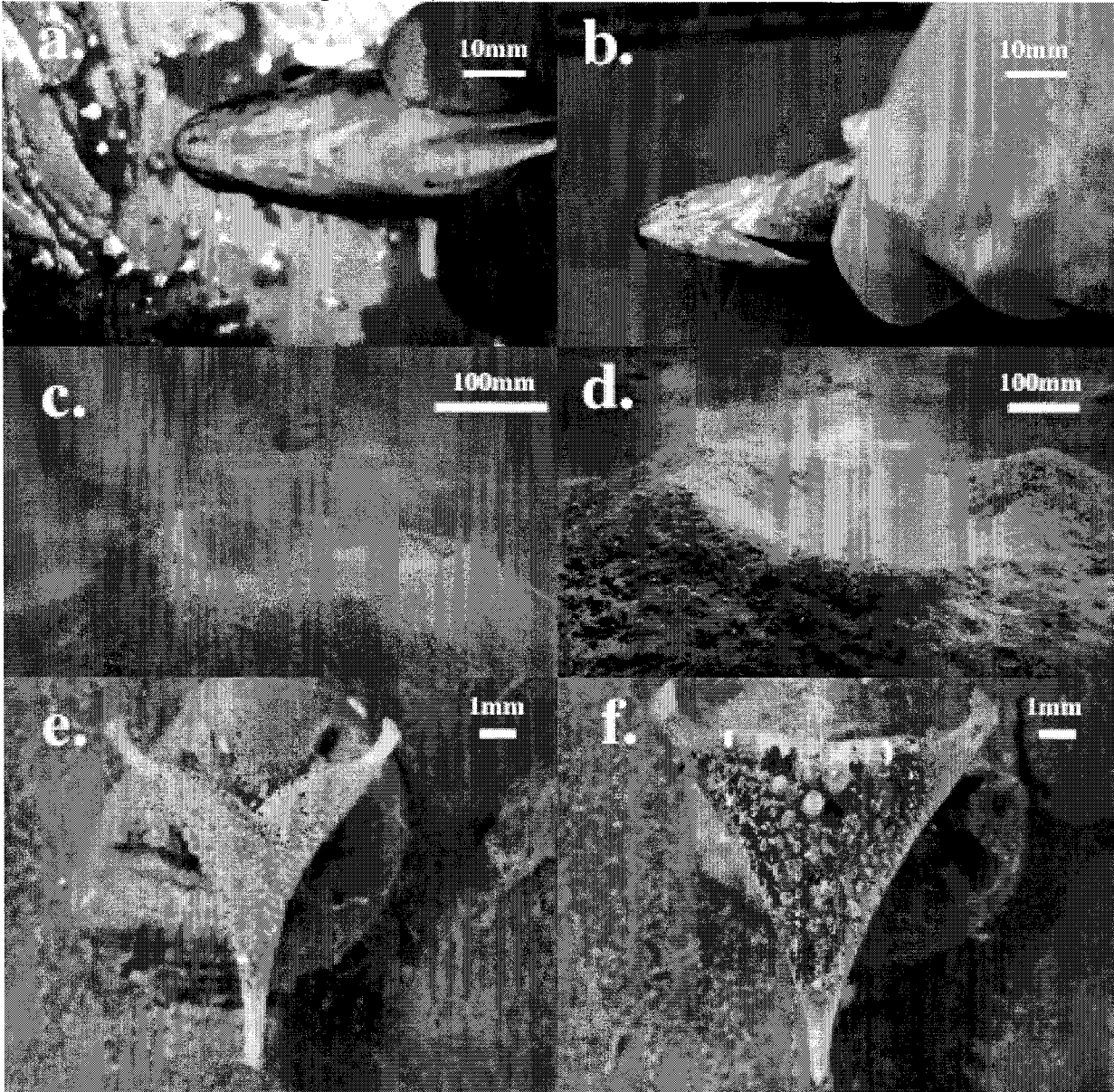
Literature Cited

Albertson R.C. (2002). *Genetic basis of adaptive morphological radiation in cichlid fishes.* PhD thesis, University of New Hampshire.

Albertson R.C., Markert J.A, Danley P.D., Kocher T.D. (1999). Phylogeny of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. *Proceedings of the National Academy of Sciences of the USA,* **96,** 5107-5110.

Albertson R.C., Kocher, T.D. (2001). Assessing morphological differences in an adaptive trait: A landmark-based morphometric approach. *Journal of Experimental Zoology,* **289,** 385-403.

Albertson, R.C., Streelman J.T. & Kocher, T.D. (2003). Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proceedings of the National Academy of Science, USA* **100,** 5252-5257.

Albertson, R.C., Streelman, J.T., Kocher, T.D., Yelick, P.C. (2005). Intergration and evolution of the cichlid mandible: The molecular basis of alternate feeding strategies. *Proceedings of the National Academy of Sciences* **102,** 16287-16292.

Alfaro, M., Bolnick, D.I., Wainwright, P.C. (2004). Evolutionary Dynamics of Complex Biomechanical Systems: an Example using the Four-Bar Mechanism. *Evolution* **58,** 495-503.

Allender, C.J., Seehausen O., Knight M.E., Turner G.F., Maclean N. (2003). Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. *Proceedings of the National Academy of Sciences of the USA,* **100,** 14074-14079.

Amorim, M. C. P., Knight, M. E., Stratoudakis, Y., & Turner, G. F. (2004). Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. *Journal of Fish Biology* **65,** 1358-1371.

Amundsen, T. & Forsgren, E. (2003). Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology* **54,** 55-64.

Andersson, M. B. (1994). *Sexual Selection.* Princeton, NJ, Princeton University Press.

Arnegard M.E., Snoeks J. (2001). New three-spotted cichlid species with hypertrophied lips (Teleostei: Cichlidae) from the deep waters of Lake Malawi/Nyasa, Africa. *Copeia* **2001,** 705-717.

Arnegard M.E., Kondrashov A.S. (2004). Sympatric speciation by sexual selection alone is unlikely. *Evolution* **58,** 222-237.

Baerends, G. P. & Baerends-van Roon, J. M. (1950). An introduction to the study of the ethology of cichlid fishes. *Behaviour*, suppl. 1, 1-242.

Baltz, D.M. & Moyle, P.B. (1993). Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications*, 3, 246-255.

Bandelt H.-J., Dress A.W.M. (1992). Split decomposition: A new and useful approach to phylogenetic analysis of distance data. *Molecular Phylogeneitcs and Evolution*, 1, 242-252.

Barel C.D.N, van Oijen M.J.P., Witte F., Witte-Maas E.L.M. (1977). An introduction to the taxonomy and morphology of the Haplochromine Cichlidae from Lake Victoria. *Netherlands Journal of Zoology*, 27, 333-389.

Barlow, G. W. (1973). Competition between Color Morphs of the Polychromatic Midas Cichlid Cichlasoma citrinellum. *Science*, 179, 806-807.

Barlow, G. W. (1989). Choosy Fishes: Mating and Related Issues. In *Ethoexperimental Approaches to the Study of Behavior* (Blanchard, R. J., Brain, P. F., Blanchard, D. C. & Parmigiani, S. eds.), pp. 267-287. Dordrecht, Kluwer Academic Publishers.

Barlow, G. W. (2002). How behavioural studies contribute to the species problem: a piscine perspective. *Fish and Fisheries*, 3, 197-212.

Barlow, G.W. (2000). The Cichlid Fishes: Nature's grand experiment in evolution. Cambridge, Massachusetts: Perseus Publishing.

Barlow, G.W. & Ballin, P.J. (1976). Predicting and assessing dominance from size and colouration in the polychromatic Midas cichlid. *Animal Behaviour*, 24, 793-813.

Barlow, G. W. & Siri, P. (1997). Does sexual selection account for the conspicuous head dimorphism in the Midas cichlid? *Animal Behaviour* 53, 573-584.

Barluenga M., Meyer A. (2004). The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? *Molecular Ecology* 13, 2061-2076.

Barraclough T.G., Vogler A.P., Harvey P.H. (1998) Revealing the factors that promote speciation. *Philosophical Transactions of the Royal Society London Series B*, 353, 241-249.

Barraclough T.G., Harvey P.H., Nee S. (1995) Sexual selection and taxanomic diversity in passerine birds. *Proceedings of the Royal Society London Series B: Biological Sciences*, 259, 211-215.

Beeching, S.C. (1995). Color pattern and inhibition of aggression in the cichlid fish *Astronotus ocellatus. Journal of Fish Biology* 47, 50-58.

158

Beeching, S. C. & Hopp, A. B.   (1999). Male mate preference and size-assortive pairing in the convict cichlid. *Journal Fish Biology* **55**, 1001-1008.

Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends in Ecology and Evolution* **17**, 571-577.

Bowers N., Stauffer J.R. Jr. (1997) Eight new species of rock-dwelling cichlids of the genus *Melanochromis* (Teleostei: Cichlidae) from Lake Malawi, Africa. *Ichthyological Exploration of Freshwaters*, **8**, 49-70.

Brooks, R. & Endler, J. A. (2001). Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**, 1644-1655.

Case, T.J. (1991). Invasion resistance, species build-up and community collapse in metapopulation models with interspecies competition. *Biological Journal of the Linnean Society*, **42**, 239-266.

Cassens I., Van Waerebeek K., Best P.B., *et al.* (2003) The phylogeography of dusky dolphins (*Lagenorhynchus obscurus*): a critical examination of network methods and rooting proceedures. *Molecular Ecology*, **12**, 1781-1792.

Chien, A. K. (1973). Reproductive behaviour of the Angel Fish *Pteophyllum scalare* (Pisces. Cichlidae). II. Influence of male stimuli upon the spawning rate of females. *Animal Behaviour*, **21**, 457-463.

Clement, T. S., Grens, K. E. & Fernald, R. D. (2005). Female affiliative preference depends on reproductive state in the African cichlid fish, *Haplochromis burtoni*. *Behavioral Ecology* **16**, 83-88.

Couldridge, V.C.K. (2002). Experimental manipulation of male eggspots demonstrates female preference for one large spot in *Pseudotropheus lombardoi*. *Journal of Fish Biology* **60**, 726-730.

Couldridge, V. C. K. & Alexander, G. J. (2001). Does the time spent near a male predict female mate choice in a Malawian cichlid? *Journal of Fish Biology* **59**, 667-672.

Couldridge, V. C. K. & Alexander, G. J. (2002). Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behavioral Ecology* **13**, 59-64.

Crapon de Caprona, M.-D. (1980). Olfactory communication in a cichlid fish, *Haplochromis burtoni*. *Zeitschrift für Tierpsychologie* **52**, 113-134.

Crapon de Caprona, M.-D. & Fritzsch, B. (1984). Interspecific fertile hybrids of Haplochromine Cichlidae (Teleostei) and their possible importance for speciation. *Netherlands Journal of Zoology* **34**, 503-538.

159

Coyne J.A., Orr, H.A. (2004) Speciation, 1st edn. Sinauer Associates, Inc., Sunderland, Massachusetts.

Danley, P.D., Markert, J.A., Arnegard, M., & Kocher, T.D. (2000) Divergence with gene flow in the rock-dwelling cichlids of Lake Malawi. *Evolution*, **54**, 1725-1737.

Danley, P. D. (2001). The ecological, behavioral, and genetic factors influencing the diversification of Lake Malawi's rock-dwelling cichlids. Ph.D. Thesis. Durham, University of New Hampshire.

Danley, P.D., Kocher, T.D. (2001). Speciation in rapidly diverging systems: lessons form Lake Malawi. *Molecular Ecology*, **10**, 1075-1086.

Darwin, C. (1871). The Descent of Man, and Selection in Relation to Sex. J. Murray; London.

Deutsch, J. C. 1997. Colour diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? *Biological Journal of the Linnean Society* **62**, 1-14.

Dick, J.T.A., Elwood, R.W., & Montogomery, W.I. (1995), The behavioural basis of a species replacement: Differential aggression and predation between the introduced *Gammarus pulex* and the native *G. duebeni celticus* (Amphipoda). *Behavioral Ecology and Sociobiology*, **37**, 393-398.

Dieckmann, U., Doebeli, M. (1999). On the origin of species by sympatric speciation. *Nature*, **400**, 354-357.

Dijkstra, P.D., Seehausen, O., Groothuis, T.G.G. (2005). Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behavioral Ecology and Sociobiology*, **58**, 136-143.

Dobzhansky, T. (1940). Speciation as a stage in evolutionary divergence. *American Naturalist* **74**, 312-321.

Dominey, W. J. (1984). Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. In: *Evolution of Fish Species Flocks* (Echelle, A. A. & Kornfield, I. eds.), pp. 231-249. Orono, Maine: Univeristy of Maine at Orono Press.

Dosen, L. D. & Montgomerie, R. (2004). Female size influences mate preferences of male guppies. *Ethology* **110**, 245-255.

Duponchelle, F., Ribbink, A.J. (2000). *Fish Ecology Report. Lake Malawi/Nyasa/Niassa Biodiversity Conservation Project.* SADC/GEF (Southern African Development Community, Gaborone, Botswana/ Global Environmental Facility), Washington, D.C.

Eccles, D.H. (1974). An outline of the physical limnology of Lake Malawi (Lake Nyasa). *Limnology and Oceanography*, **19**, 730-742.

160

Eccles, D.H., Lewis, D.S.C. (1978). A taxonomic study of the genus *Lethrinops* Regan (Pisces-Cichlidae) from Lake Malawi, part 2. *Ichthyological Bulletin of the J. L. B. Smith Institute of Ichthyology*, **37**, 1-11.

Eccles, D.H., Lewis, D.S.C. (1979). A taxonomic study of the genus *Lethrinops* Regan (Pisces-Cichlidae) from Lake Malawi, part 3. *Ichthyological Bulletin of the J. L. B. Smith Institute of Ichthyology*, **38**, 1-25.

Eccles, D.H., Trewavas, E. (1989). *Malawian Cichlid Fishes. The classification of some Haplochromine genera*. Lake Fish Movies, Herten, West Germany.

Elton, C.S. (1958). The Ecology of Invasions by Animals and Plants. London: Menthuen.

Enserink, M. (1999). Biological invaders sweep in. *Science*, **285**, 1834-1836.

Evans, M.R. & Norris, K. (1996). The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids (*Cichlasoma meeki*). *Behavioral Ecology* **7**, 1-6.

Falter, U. & Charlier, M. (1989). Mate choice in pure-bred and hybrid females of *Oreochromis niloticus* and *O. mossambicus* based upon visual stimuli. *Biology of Behaviour* **14**, 265-276.

Forsgren, E. (1992). Predation risk affects mate choice in a Gobiid fish. *American Naturalist* **140**, 1041-1049.

Fryer, G. (1959). The Trophic Interrelationships and Ecology of Some Littoral Communities of Lake Nyasa with Especial Reference to the Fishes, and a Discussion of the Evolution of a Group of Rock-Frequenting Cichlidae. *Proceedings of the Zoological Society, LondonI*, **132**, 153-281.

Fryer, G. & Iles, T. D. (1972). *The Cichlid Fishes of the Great Lakes of Africa, Their Biology and Evolution*. Neptune City, T.F.H..

Fuller, R. C. (2003). Disentangling female mate choice and male competition in the Rainbow Darter, *Etheostoma caeruleum*. *Copeia* **200**, 138-148.

Gabor, C. (1999). Association patterns of Sailfin Mollies (*Poecilia latipinna*): alternative hypothesis. *Behavioral Ecology and Sociobiology* **46**, 333-340.

Gabor, C. R. & Ryan, M. J. (2001). Geographical variation in reproductive character displacement in mate choice by male sailfin mollies. *Proceedings of the Royal Society London, Series B* **268**, 1063-1070.

Gamradt, S.C., Katz, L.B., & Anzalone, C.B. (1997). Aggression by non-native crayfish deters breeding in California newts. *Conservation Biology*, **11**, 793-796.

Genner, M.J. & Turner, G.F. (2005). The mbuna cichlids of Lake Malawi: A model for rapid speciation and adaptive radiation. *Fish and Fisheries*, **6**, 1-34.

Genner MJ, George F. Turner and Stephen J. Hawkins (1999a) Resource control by territorial male cichlid fish in Lake Malawi. *Journal of Animal Ecology* **68**, 522-529.

Genner, M.J., Turner, G.F., Hawkins, S.J. (1999b). Foraging of rocky habitat cichlid fishes in Lake Malawi: coexistence through niche partitioning? *Oecologia*, **121**, 283-292.

Genner, M.J., Seehausen, O., Cleary, D.F.R., *et al.* (2004) How does the taxonomic status of allopatric populations influence species richness within African cichlid fish assemblages? *Journal of Biogeography,* **31**, 93-102.

Goldschmidt, T. & deVisser, J. (1990). On the Possible Role of Egg Mimics in Speciation. *Acta Biotheretical,* **38**, 125-134.

Greenwood, P.H. (1984). African Cichlids and Evolutionary Theories. In: *Evolution of Fish Species Flocks* (eds. Echelle AA, Kornfield I), pp. 141-154. University of Maine at Orono Press, Orono, Maine.

Hankison, S. J. & Morris, M. R. (2003). Avoiding a compromise between sexual selection and species recognition: female Swordtail Fish assess multiple species-specific cues." *Behavioral Ecology* **14**, 282-287.

Hay, D. E. & McPhail, J. D. (1975). Mate selection in Threespine Sticklebacks (Gasterosteus). *Canadian Journal of Zoology* **53**, 441-450.

Hert, E. (1986). On the Significance of the Egg-Spots in an African Mouth-Brooder (Haplochromis elegans, Trewavas 1933). *Ann. Kon. Mus. Mid. Afr. Zool. Wetensch.* **251**, 25-26.

Hert, E. (1989). The function of egg-spots in an African mouth-brooding cichlid fish. *Animal Behaviour* **37**, 726-732.

Hert, E. (1990). Factors in habitat partitioning in *Pseudotropheus aurora* (Pisces: Cichlidae), an introduced species to a species-rich community of Lake Malawi. *Journal of Fish Biology*, **36**, 853-865.

Hert, E. (1991). Female choice based on egg-spots in Pseudotropheus aurora Burgess 1976, a rock-dwelling cichlid of Lake Malawi, Africa. *Journal of Fish Biology*, **38**, 951-953.

Hert, E. (1995). The impact of intralacustrine introductions with regard to space utilization and competition for territories to a cichlid fish community in Lake Malawi, Africa. *Ecological Research*, **10**, 117-124.

Higashi, M., Takimoto, G., & Yamamura, N. (1999). Sympatric speciation by sexual selection. *Nature* **402**, 523-525.

162

Higgins, S.I., Richardson, D.M., Cowling, R.M., & Trinder-Smith, T.H. (1999). Predicting the landscape-scale distribution of alien plants and their threat to plant diversity. *Conservation Biology*, **13**, 303-313.

Hill, B.J. & Ribbink, A.J. (1978). Depth equilibration of a shallow-water cichlid fish. *Journal of Fish Biology*, **13**, 741-745.

Hilliday, T. R. (1983). The study of mate choice. In *Mate Choice* (Bateson, P. ed.), pp. 3-32. Cambridge, Cambridge University Press

Hillis, D.M., Huelsenbeck, J.P. (1992). Signal, noise, and reliability in molecular phylogenetic analysis. *Journal of Heredity*, **83**, 189-195.

Holway, D.A. & Suarez, A.V. (1999). Animal behavior: An essential component of invasion biology. *Trends in Ecology and Evolution*, **14**, 328-330.

Holzberg, S. (1978). A field and laboratory study of the behavior and ecology of *Psuedotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces; Cichlidae). *Zeitschrift fur zoologische Systematik und Evolutionsforschung* **16**, 171-187.

Hong, Y., Chuah, A. (2003). A format for databasing and comparison of AFLP fingerprint profiles. *BMC Bioinformatics*, **4**: 7.

Hoogerhoud, R.J.C. (1984). A taxonomic reconsideration of the Haplochromine genera *Gaurochromis* Greenwood 1980 and *Labrochromis* Regan 1920 (Pisces, Cichlidae). *Netherlands Journal of Zoology*, **34**, 539-565.

Hori, M. (1997). Structure of Littoral Fish Communities Organized by Their Feeding Activities. In: *Fish Communities in Lake Tanganyika* (ed. Kawanabe H, Michio Hori and Makoto Nagoshi), pp. 277-298. Kyoto University Press, Sakyo-ku, Kyoto.

Hudson, D.H. (1998). Splits Tree: analyzing and visualizing evolutionary data. *Bioinformatics*, **14**, 68-73.

Hurd, P.L. (1997). Cooperative signalling between opponents in fish fights. *Animal Behaviour* **54**, 1309-1315.

Huysseune, A. (1995). Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatoreochromis alluaudi* (Teleostei: Cichlidae). *Archives of Oral Biology*, **40**, 1005-1014. Jordan, R., Kellogg, K., Juanes, F., & Stauffer, J. Jr. (2003). Evaluation of female mate choice cues in a group of Lake Malawi *Mbuna* (Cichlidae). *Copeia* **2003**, 181-186.

Jordan, R., Kellogg, K., Juanes, F., Stauffer, J. Jr. (2003). Evaluation of Female Mate Choice Cues in a Group of Lake Malawi *Mbuna* (Cichlidae). *Copeia* **2003**, 181-186.

Kassam, D., Seki, S., Rusuwa, B., Ambali, A.J.D., Yamaoka, K. (2005). Genetic diversity within the genus Cynotilapia and its phylogenetic position among Lake Malawi's mbuna cichlids. *African Journal of Biotechnology* **4**, 1195-1202.

Kaufman, L. (1992). Catastrophic change in species-rich freshwater ecosystems. *Bioscience*, **42**, 846-858.

Kellogg, K., Markert, J. A., Stauffer, J. R., Jr., & Kocher, T. D. (1995). Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proceedings of the Royal Society, London Series B* **260**, 79-84.

Kellogg, K. (1997). Lake Malawi cichlid mating systems, factors that influence mate selection. PhD Thesis, Pennsylvania State University.

Kellogg, K. A., Stauffer, J. R. Jr., & McKaye, K. R. (2000). Characteristics that influence male reproductive success on a lek of *Lethrinops* c.f. *parvidens* (Teleostei, Cichlidae). *Behavioural Ecology and Sociobiology* **47**, 164-170.

Kidd, M.R., Danley, P.D., Kocher, T.D. (2006a). A direct assay of female choice in cichlid fishes: All the eggs in one basket. *Journal of Fish Biology* **68**, 373-384.

Kidd, M.R., Kidd, C. E., Kocher, T. D. (2006b). Axes of differentiation in the bower-building cichlids of Lake Malawi. *Molecular Ecology* **15**, 459-478.

Kirkpatrick, M. (1987). Sexual selection by female choice in polygynous animals. *Annual Review of Ecology and Systematics* **18**, 43-70.

Kirkpatrick, M. & Ravigné, V. (2002). Speciation by natural and sexual selection: models and experiments. *American Naturalist* **159**, S22-S35.

Kirkpatrick, M., Nuismer, S.L. (2004). Sexual selection can constrain sympatric speciation. *Proceedings of the Royal Society London Series B: Biological Sciences*, **271**, 687-693.

Kishino, H., Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topology from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution*, **29**, 170-179.

Knight, M. E., Turner, G. F., Rico, C., van Oppen, M. J. H., & Hewett, G. M. (1998). Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Molecular Ecology* **7**, 1605-1610.

Knight, M. E. & Turner, G. F. (1999). Reproductive isolation among closely related Lake Malawi cichlids: can males recognize females by visual cues? *Animal Behaviour* **58**, 761-768.

Knight, M.E., van Oppen, M.J.H., Smith, H.L., Rico, C., Hewitt, G.M., & Turner, G.F. (1999). Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology*, **8**, 1521-1527.

Knight, M. E. & Turner, G. F. (2004). Laboratory mating trials indicate incipient speciation by sexual selection among populations of the cichlid fish *Pseudotropheus zebra* from Lake Malawi. *Proceedings of the Royal Society, London Series B* **271**, 675-680.

Kodric-Brown, A. (1993). Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behavioural Ecology and Sociobiology* **32**, 415-420.

Kocher, T.D. (2003). Fractious phylogenies. *Nature* **423**, 489-491.

Kocher, T.D. (2004). Adaptive evolution and explosive speciation: The cichlid fish model. *Nature Reviews Genetics*, **5**, 288-298.

Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R. Jr. (1993). Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution*, **2**, 158-165.

Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R. Jr., Lockwood, S.F. (1995). Evolution of NADH Dehydrogenase subunit 2 in East African cichlid fish. *Molecular Phylogenetics and Evolution*, **4**, 420-432.

Kohda, M. (1998). Coexistence of permanently territorial cichlids of the genus Petrochromis through male-mating attack. *Environmental Biology of Fishes*, **52**, 231-242.

Kolar, C.S. & Lodge, D.M. (2001). Progress in invasion biology: Predicting invaders. *Trends in Ecology and Evolution*, **16**, 199-204.

Kondrashov, A. S. & Kondrashov, F. A. (1999). Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400**, 351-354.

Konings, A. (1990). Descriptions of six new Malawi cichlids. *Tropical Fish Hobbyist*, **38**, 110-129.

Konings, A. (2000). Exploring Lake Malawi: The cichlids of Thumbi West Island. *Cichlid News*, **9**, 6-13.

Konings, A. (2001). Malawi Cichlids in their Natural Habitat. El Paso, TX: Cichlid Press.

Kornfield, I. & Taylor, J.N. (1983). A new species of polymorphic fish, *Cichlasoma minckleyi* from Cuarto Cienegas, Mexico (Teleostei: Cichlidae). *Proceedings of the Biological Society of Washington*, **96**, 253-269.

Kornfield, I. & Parker, A. (1997). Molecular Systematics of a Rapidly Evolving Species Flock: The mbuna of Lake Malawi and the Search for Phylogenetic Signal. In: *Molecular Systematics of Fishes* (ed. Kocher, T. & Stepien, C.A.), pp. 25-37. Academic Press, San Diego.

Kornfield, I. & Smith, P. F. (2000). African cichlid fishes, model systems for evolutionary biology. *Annual Review of Ecology and Systematics* **31**, 163-196.

Kosswig, C. (1947), Selective mating as a factor for speciation in cichlid fish of East African lakes. *Nature* **159**, 604-605.

Kusmierski, R., Borgia, G., Uy, A., Crozier, R.H. (1997). Labile evolution of display traits in Bowerbirds indicates reduced effects of phylogenetic constraint. *Proceedings of the Biological Society of Washington*, **264**, 307-313.

Kusmierski, R., Borgia, G., Crozier, R.H., Chan, B.H.Y. (1993). Molecular information on bowerbird phylogeny and the evolution of exaggerated male characteristics. *Journal of Evolutionary Biology*, 6:737-752.

Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences of the USA*, **78**, 3721-3725.

Lande, R. (1982). Rapid origin of sexual isolation and character divergence in a cline. *Evolution*, **36**, 213-223.

Lande, R., Seehausen, O., & van Alphen, J. J. M. (2001). Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. *Genetica* **112-113**, 435-443.

Lanzing, W.J.R. & Bower, C.C. (1974). Development of colour patterns in relation to behaviour in *Tilapia mossambica* (Peters). *Journal of Fish Biology*, **6**, 29-41.

Lewis, D.S.C. (1982). A revision of the genus *Labidochromis* (Teleostei: Cichlidae) from Lake Malawi. *Zoological Journal of the Linnean Society*, **75**, 189-265.

Lewis, D.S.C. (1982). Problems of Species Definitions in Lake Malawi Cichlid Fishes (Pisces: Cichlidae). *J. L. B. Smith Institute of Ichthyology Special Publication* **23**, 1-5.

Lewis, D.S.C., Reinthal, P., Trendall, J. (1986). *A guide to the fishes of Lake Malawi National Park*. World Wildlife Fund, Gland, Switzerland.

Liem, K.F., Kaufman, L.S, (1984). Intraspecific macroevolution: Functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. In: *Evolution of Fish Species Flocks* (eds. Echelle AA, Kornfield I), pp. 203-215. University of Maine at Orono Press, Orono, Maine.

Lobel, Philip S. (1998). Possible species specific courtship sounds by two sympatric cichlid fishes in Lake Malawi, Africa. *Environmental Biology of Fishes* **52**, 443-452.

Lodge, D.M. (1993). Biological invasions: Lessons for ecology. *Trends in Ecology and Evolution*, **8**, 133-137.

Losos, J.B. (2000). Ecological Character Displacement and the Study of Adaptation. *Proceedings of the National Academy of Sciences*, **97**, 5693-5695.

Losos, J.B. & Glor, R.E. (2003). Phylogenetic Comparative Methods and the Geography of Speciation. *Trends in Ecology and Evolution* **18**, 220-227.

Louda, S.M., Gray, W.N., McKaye, K.R., Mhone, O.J. (1983). Distribution of Gastropod genera over a vertical depth gradient at Cape Maclear, Lake Malawi. *Veliger*, **25**, 387-392.

Ludlow, A. M., Itzkowitz, M. & Baird, D. R. (2001). Male mating success and female preference for multiple traits in the Twoline Pupfish (*Cyprinodon bifasciatus*). *Behaviour* **138**, 1303-1318.

Maan, M.E., Seehausen, O., Söderberg, L., Johnson, L., Ripmeester, E. A., Mrosso, H.D.J., Taylor, M.I., van Dooren, T.J.M., van Alphen J.J.M. (2004). Intraspecific sexual selection on a speciation trait, male coloration, in the Lake Victorian cichlid *Pundamilia nyererei*. *Proceedings of the Royal Society London, Series B*, **271**, 2445-2452.

Maddison, D.R. & Maddison, W.P. (2003). MacClade. Sinauer Associates, Inc., Sunderland, Massachusetts.

Marchetti, M.P., Moyle, P.B., & Levene, R. (2004). Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. *Freshwater Biology*, **49**, 646-661.

Markert, J.A., Danley, P.D., & Arnegard, M.E. (2001). New markers for new species: microsatellite loci and the Eash African cichlids. *Trends in Ecology and Evolution*, **16**, 100-107.

Marsh, A.C. & Ribbink, A.J. (1981). A comparison of the abilities of three sympatric species of Petrotilapia (Cichlidae, Lake Malawi) to penetrate deep water. *Environmental Biology of Fishes*, **6**, 367-369.

Marsh, B. A., Marsh, A. C., & Ribbink, A. J. (1986). Reproductive seasonality in a group of rock-frequenting cichlid fishes in Lake Malawi. *Journal of Zoology* **209**, 9-20.

Mayr. E. (1963). *Animal Species and Evolution*. Belknap, Cambridge, MA.

Mayr, E. (1984). Evolution of Fish Species Flocks: A Commentary. In: *Evolution of Fish Species Flocks* (ed. Echelle, A.A. & Kornfield, I.), pp. 3-12. University of Maine at Orono Press, Orono, Maine.

McElroy, D. M. & Kornfield, I. (1990). Sexual selection, reproduction behavior, and speciation in the mbuna species flock of Lake Malawi. *Environmental Biology of Fishes* **28**, 273-284.

167

McKaye, K.R. (1977). Competition for breeding sites between the cichlid fishes of Lake Jiloa, Nicaragua. *Ecology* **58**, 291-302.

McKaye, K.R. (1983). Ecology and breeding behavior of a cichlid fish, *Cyrtocara eucinostomus*, on a large lek in Lake Malawi, Africa. *Environmental Biology of Fishes*, **8**, 81-96.

McKaye, K.R. (1984). Behavioural aspects of cichlid reproductive strategies: Patterns of territoriality and brood defense in Central American substratum spawners and African mouth brooders. In: *Fish Reproduction: Strategies and Tactics* (eds. Potts GW, Wootton RJ), pp. 245-273. Academic Press, London.

McKaye, K.R. (1991). Sexual selection and the evolution of the cichlid fishes of Lake Malawi, Africa. In: *Cichlid Fishes: Behaviour, ecology, and evolution* (ed. Keenleyside, MHA), pp. 241-257. Chapman & Hall, London.

McKaye, K. R., Louda, S. M., and Stauffer, J. R., Jr. (1990). Bower size and male reproductive success in a cichlid fish lek. *American Naturalist* **135**, 597-613.

McKaye, K.R., Howard, J.H., Stauffer, J.R. Jr., Morgan, R.P., Shonhiwa, F. (1993). Sexual selection and genetic relationships of a sibling species complex of bower building cichlids in Lake Malawi, Africa. *Japanese Journal of Ichthyology*, **40**, 15-21.

McKaye, K.R., Stauffer, J.R. Jr., Turner, G.F., Konings, A., Sato, T. (2001). Fishes, as well as birds, build bowers. *Journal of Aquariculture and Aquatic Sciences*, **9**, 121-133.

McKaye, K.R., Stauffer, J.R. Jr, Van Den Berghe, E.P., *et al.* (2002) Behavioral, morphological and genetic evidence of divergence of the Midas cichlid species complex in two Nicaraguan crater lakes. *Cuadernos de la Investigacion de la UCA*, **12**, 19-47.

Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implication for speciation in cichlid fishes. *Evolution*, **41**, 1357-1369.

Meyer, A. (1993). Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology and Evolution*, **8**, 279-284.

Meyer, A., Kocher, T.D., Basasibwaki, P., Wilson, A.C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550-553.

Moran, P., Kornfield, I. (1993). Retention of an ancestral polymorphism in the Mbuna species flock (Teleostei: Cichlidae) of Lake Malawi. *Molecular Biology and Evolution*, **10**, 1015-1029.

Moran, P., Kornfield, I., Reinthal, P.N. (1994). Molecular systematics and radiation of the Haplochromine cichlids (Teleostei: Perciformes) of Lake Malawi. *Copeia*, **1994**, 274-287.

Moretz, J.A., & Morris, M.R. (2003) Evolutionarily labile responses to a signal of aggressive intent. *Proceedings of the Royal Society London, Series B*, **270**, 2271-2277.

Morris, M. R., Batra, P., and Ryan, M. J. (1992). Male-male competition and access to females in the swordtail *Xiphophorus nigrensis*. *Copeia* 1992, 980-986.

Morris, M.R., Mussel, M.,. Ryan, M. J. (1995). Vertical bars on male *Xiphophorus multilineatus*: a signal that deters rival males and attracts females. *Behavioral Ecology* **6**, 274-279.

Morris, M. R., Nicoletto, P. F. & Hesselman, E. (2003). A polymorphism in female preference for a polymorphic male trait in the Swordtail Fish *Xiphophorus cortezi*. *Animal Behaviour* **65**, 45-52.

Moyle, P.B. (1999). Effects of invading species on freshwater and extuarine ecosystems. In: *Invasive species and biodiversity management*, (eds. O.T. Sandlund, P.J. Schei, & A. Viken), pp. 177-191. Netherlands: Kluwer Academic Press.

Moyle, P.B. & Light T. (1996). Fish invasions in California: Do abiotic factors determine success? *Ecology*, **77**, 1666-1670.

Munthali, S.M. (1997). Dwindling food-fish species and fishers' preference: problems of conserving Lake Malawi's biodiversity. *Biodiversity and Conservation*, **6**, 253-261.

Munthali, S.M. & Ribbink, A.J. (1998). Condition and fecundity of translocated rock-dwelling cichlid fish in Lake Malawi. *Journal of Zoology*, **244**, 347-355.

Nagel, L. & Schluter, D. (1998). Body size, natural selection, and speciation in Sticklebacks. *Evolution* **52**, 209-218.

Nei, M., Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA*, **76**, 5269-5273.

Nelson, C. M. (1995). Male size, spawning pit size and female mate choice in a lekking cichlid fish. *Animal Behaviour* **50**, 1587-1599.

Ngatunga, B.P., Snoeks, J. (2003). *Lethrinops turneri*, a new shallow-water haplochromine cichlid (Teleostei: Cichlidae) from the Lake Malawi basin. *Ichthyological Exploration of Freshwaters*, **14**, 127-136.

Orr, M.R., Smith, T.B. (1998). Ecology and speciation. *Trends in Ecology and Evolution*, **13**, 502-506.

Owen, R.B., Crossley, R., Johnson, T.C., Tweddle, I., Kornfield, I., Davision, S., Eccles, D.H., & Engstrom, D.E. (1990). Major low levels of Lake Malawi and their implications for speciation rates in cichlid fishes. *Proceedings of the Royal Society London, Series B*, **240**, 519-553.

Panhuis, T.M., Butlin, R., Zuk, M., Tregenza, T. (2001). Sexual selection and speciation. *Trends in Ecology and Evolution*, **16**, 364-371.

Parker, A. & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes* **47**, 345-352.

Parker, A, Kornfield, I. (1997). Evolution of the mitochondrial DNA control region in the mbuna (Cichlidae) species flock of Lake Malawi, East Africa. *Journal of Molecular Evolution*, **45**, 70-83.

Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Willaimson, M.H., Von Holle, B., Moyle, P.B., Byers, J.E., & Goldwasser, L. (1999). Impact: Toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**, 3-19.

Pauers, M.J., McKinnon, J.S., Ehlinger, T.J. (2004). Directional sexual selection on chroma and within-pattern colour contrast in Labeotropheus fuelleborni. *Proceedings of the Royal Society London, Series B, Biology Letters* **Suppl.**, Online.

Pereyra, R., Taylor, M.I., Turner, G.F., Rico, C. (2004). Variation in habitat preference and population structure among three species of the Lake Malawi cichlid genus *Protomelas*. *Molecular Ecology*, **13**, 2691-2697.

Perrins, J., Fitter, A, & Williamson, M. (1993). Population biology and rates of invasion of three introduced *Impatiens* species in the British Isles. *Journal of Biogeography*, **20**, 33-44.

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience*, **50**, 53-65.

Plenderleith, M., van Oosterhout, C., Robinson, R.L., Turner, G.F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters*, **1**, 411-414.

Podos, J. (2001). Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185-188.

Poll, M. (1986). Classification des Cichlidae du lec Tanganyika. *Memoires de la classe des sciences, Academie royale de Beligique*, **45**, 1-163.
Posada, D., Crandall, K.A. (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37-44.

Ptacek, M. B. (1998). Interspecific mate choice in sailfin and shortfin species of mollies. *Animal Behaviour* **56**, 1145-1154.

Ptacek, M. B. (2000). The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behavioural Processes* **51**, 111-134.

Rahel, F.J. (2000). Homogenization of fish faunas across the United States. *Science*, **288**, 854-856.

Regan, C.T. (1921). The cichlid fishes of Lake Nyassa. *Proceedings of the Zoological Society of London*, **1921**, 675-727.

Reinthal, P. (1990). The feeding habits of a group of herbivorous rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa. *Environmental Biology of Fishes*, **27**, 215-233.

Reinthal, P. (1993). Evaluating biodiversity and conserving Lake Malawi's cichlid fish fauna. *Conservation Biology*, **7**, 712-718.

Reinthal, P.N, & Meyer, A. (1997). Molecular phylogentic tests of speciation models in Lake Malawi cichlid fishes. In: *Molecular evolution and adaptive radiation* (eds. Givnish TJ, Sytsma KJ), pp. 375-390. Cambridge University Press, Cambridge.

Ribbink, A. J., Marsh, B. A., Marsh, A. C., Ribbink, A. C., & Sharp, B. J. (1983). A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology* **18**, 149-310.

Ribbink, A.J., Barber, D.G., Bootsma, H.A., *et al.* (2001). The Lake Malawi/Niassa/Nyasa biodiversity conservation programme: Science in a socio-economic context. In: *African fish and fisheries - diversity and utilization* (ed. Skelton PH, Teugels GG), pp. 28-59. Royal Museum for Central Africa, Tervuren, Belgium.

Ricciardi, A. & Rasmussen, J.B. (1998). Predicting the identity and impact of future biological invaders: A priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Science*, **55**, 1759-1765.

Rice, A. N. and Lobel, P. S. (2002). Enzyme activities of pharyngeal jaw musculature in the cichlid *Tramitichromis intermedius*, implications for sound production in cichlid fishes. *Journal of Experimental Biology* **205**, 3519-3523.

Rohlf, F. J. (1993). Relative warp analysis and an example of its application to mosquito wings. Pp. 134–159 *in* L. F. Marcus, E. Bello, and A. Garcia-Valdecasas, eds. Contributions to morphometrics. CSIC, Madrid.

Rosecchi, E., Thomas, F., & Crivelli, A.J. (2001). Can life-history traits predict the fate of introduced species? A case study on two cyprinid fish in southern France. *Freshwater Biology*, **46**, 845-853.

Rossiter, A., Yamagishi, S. (1997). Intraspecific plasticity in the social system and mating behaviour of a lek-breeding cichlid fish. In: *Fish Communities in Lake Tanganyika* (eds. Kawanabe H, Hori M, Nagoshi M), pp. 195-217. Kyoto University Press, Sakyo-ku, Kyoto.

Rundle, H.D., Nagel, L., Boughman, J.W., Schluter, D. (2000). Natural selection and parallel speciation in sympatric sticklebacks. *Science*, **287**, 306-307.

Rundle, H.D., Nosil, P. (2005). Ecological speciation. *Ecology Letters*, **8**, 336-352.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Mollfsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., & Weller, S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305-332.

Salzburger, W., Meyer, A., Baric, S., Verheyen, E., Sturmbauer, C. (2002). Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. *Systematic Biology*, **51**, 113-135.

Salzburger, W., Baric, B., Sturmbauer, C. (2002). Speciation via introgressive hybridization in East African cichlids? *Molecular Ecology* **11**, 619-625.

Salzburger, W., Mack, T, Verheyen, E., Meyer, A. (2005). Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology* **5**.

Salzburger, W. & Meyer, A. (2004). The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* **91**, 277-290.

Schliewen, U., Rassmann, K., Markmann, M., *et al.* (2001). Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric condition in Lake Ejagham, Cameroon. *Molecular Ecology*, **10**, 1471-1488.

Schliewen, U.K., Klee, B. (2004). Reticulate sympatric speciation in Cameroonian crater lake cichlids. *Frontiers in Zoology*, **1**, 5.

Schluter, D. & McPhail, J.D. (1993). Character Displacement and Replicate Adaptive Radiation. *Trends in Ecology and Evoluion,* **8**, 279-284.

Schroder, J.H. (1980). Morphological and behavioural differences between the BB/OB and B/W colour morphs of *Pseudotropheus zebra* Boulenger (Pisces; Cichlidae). *Zeits. Zool. Sys. Evol.* **18**, 69-76.

Scott, R. J. and Forster, S. A. (2000). Field data do not support a textbook example of convergent character displacement. *Proceedings of the Royal Society, London Series B* **267**, 607-612.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, **19**, 198-207.

172

Seehausen, O. & van Alphen, J. J. M. (1998). The effect of male colouration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyereri* complex). *Behavioural Ecology Sociobiology* **42**, 1-8.

Seehausen, O., van Alphen, J. J. M., & Witte, F. (1997). Cichlid fsh diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808-1811.

Seehausen, O., Witte, F., van Alphen, J. J. M. & Bouton, N. (1998). Direct mate choice maintains diversity among sympatric cichlids in Lake Victoria. *Journal of Fish Biology* **53**(Supplement A), 37-55.

Seehausen, O., Mayhew, P. J. & van Alphen, J. J. M. (1999). Evolution of colour patterns in East African cichlid fish. *Journal of Evolutionary Biology* **12**, 514-534.

Seehausen, O., Koetsier, E., Schneider, M.V., *et al.* (2003) Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock. *Proceedings of the Royal Society London Series B: Biological Sciences*, **270**, 129-137.

Seehausen, O. & Schluter, D. (2004). Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proceedings of the Royal Society London Series B: Biological Sciences*, **271**, 1345-1353.

Shaw, P.W., Turner, G.F., Idid, M.R., Robinson, R.L., Carvalho, G. (2000). Genetic population structure indicates sympatric speciation of Lake Malawi pelagic cichlids. *Proceedings of the Royal Society London Series B: Biological Sciences*, **267**, 2273-2280.

Shimodaira, H. & Hasegawa, M. (1999). Multiple Comparisons of Log-Likelihoods with Applications to Phylogenetic Inference. *Molecular Biology and Evolution* **16**, 1114-1116.

Slovin, M. & Rowland, W.J. (1978). The effects of color patterns on the aggressive behavior of Tilapia mariae (Boulenger). *Behavioural Biology*, **24**, 378-386.

Smith, G.R. & Todd, T.N. (1984). Evolution of Species Flocks of Fishes in North Temperate Lakes. In: *Evolution of Fish Species Flocks* (ed. Echelle AAaIK), pp. 45-68. Univeristy of Maine at Orono Press, Orono, Maine.

Smits, J.D., Witte, F., van Veen, F.G. (1996). Functional changes in the anatomy of the pharyngeal jaw apparatus of *Astatoreochromis alluaudi* (Pisces, Cichlidae), and their effects on adjacent structures. *Biological Journal of the Linnean Society*, **59**, 389-409.

Soltis, P.S., Gitzendanner, M.A. (1999). Molecular systematics and the conservation of rare species. *Conservation Biology*, **13**, 471-483.

Smith, P. F. & Kornfield, I. (2002). Phylogeography of Lake Malawi cichlids of the genus *Pseudotropheus*: significance of allopatric colour variation. *Proceedings of the Royal Society London, Series B*, **269**, 2495-2501.

173

Smith, P.F., Konings, A., Kornfield, I. (2003). Hybrid origin of a cichlid population in Lake Malawi: implications for genetic variation and species diversity. *Molecular Ecology*, **12**, 2497-2504.

Stauffer, J.R. Jr. (1993). A new species of *Protomelas* (Teleostei: Cichlidae) from Lake Malawi, Africa. *Ichthyological Exploration of Freshwaters*, **4**, 343-350.

Stauffer, J.R. Jr. & Hert, E. (1992). *Psuedotropheus callainos*, a new species of mbuna (Cichlidae), with analyses of changes associated with two intra-lacustrine transplantations in Lake Malawi, Africa. *Ichthyological Exploration of Freshwaters*, **3**, 253-264.

Stauffer, J.R. & Kellogg, K.A. (1996). Sexual selection in Lake Malawi cichlids. In: *The Cichlid Yearbook* (ed. Konings A), pp. 23-28. Cichlid Press, Ahornweg, Germany.

Stauffer, J.R. & Sato, T. (2002). A new species of *Copadichromis* (Cichlidae) from Thumbi West Island, Lake Malawi. *Ichthyological Exploration of Freshwaters*, **13**, 91-95.

Stauffer, J.R. Jr. & Gray, E. (2004). Phenotypic plasticity: its role in trophic radiation and explosive speciation in cichlids (Teleostei: Cichlidae). *Animal Biology* **54**, 137-158.

Stauffer, J. R. Jr., LoVullo, T. J. & McKaye, K. (1993). Three new sand-dwelling cichlids from Lake Malawi, Africa, with a discussion of the status of the Genus *Copadichromis* (Teleostei, Cichlidae). *Copeia* **1993**, 1017-1027.

Stauffer, J.R. Jr., Bowers, N.J., McKaye, K.R., Kocher, T.D. (1995). Evolutionarily significant units among cichlid fishes: The role of behavioral studies. *American Fisheries Society Symposium*, **17**, 227-244.

Stauffer, J.R. Jr., Bowers, N.J., Kocher, T.D., & McKaye, K.R. (1996). Evidence of hybridization between *Cynotilapia afra* and *Pseudotropheus zebra* (Teleostei: Cichlidae) following an intralacustrine translocation in Lake Malawi. *Copeia*, **1996**, 203-208.

Stauffer, J. R. J., Bowers, N. J., Kellogg, K. A. and McKaye, K. R. (1997). A revision of the blue-black *Pseudotropheus zebra* (Teleostei, Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and ten new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **148**, 189-230.

Stauffer, J.R. Jr., McKaye, K.R., Konings, A.F. (2002). Behaviour: an important diagnostic tool for Lake Malawi cichlids. *Fish and Fisheries*, **3**, 213-224.

Stiassny, M.L.J. (1991). Phylogenetic intrarelationships of the family Cichlidae: an overview. Cichlid fishes. In: *Behaviour, ecology and evolution* (ed Keenlyeside MHA), pp. 1-35. Chapman & Hall, London.

Streelman, J. T. & Danley, P. D. (2003). The stages of vertebrate evolutionary radiation. *Trends in Ecology and Evolution* **18**, 126-131.

Streelman, J.T., Webb, J. F., Albertson, R. C., Kocher, T. D. (2003). The cusp of evolution and development: a model of cichlid tooth shape diversity. *Evolution & Development* **5**, 600-608.

Streelman, J.T., Gmyrek, S.L., Kidd, M.R., Kidd, C., Robinson, R.L., Hert, E., Ambali, A.J., & Kocher, T.D. (2004). Hybridization and contemporary evolution in an introduced cichlid fish from Lake Malawi National Park. *Molecular Ecology*, **13**, 2471-2479.

Swofford, D.L. (2001). *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Ver. 4.0b8.* Sinauer Associates, Sunderland, MA.

Takahashi, K., Terai, Y., Nishida, M., Okada, N. (2001). Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika and revealed by analysis of the insertion of retroposons. *Molecular Biology and Evolution*, **18**, 2057-2066.

Takimoto, G., Higashi, M., & Yamamura, N. (2000). A deterministic genetic model for sympatric speciation by sexual selection. *Evolution* **54**, 1870-1881.

Taylor, M., Turner, G.F., Robinson, R.L., Stauffer, J.R. Jr. (1998). Sexual selection, parasites and bower height skew in a bower-building cichlid fish. *Animal Behaviour*, **56**, 379-384.

Taylor, M.I., Verheyen, E. (2001). Microsatellite data reveals weak population substructuring in *Copadichromis* sp. 'virginalis kajose', a demersal cichlid from Lake Malawi, Africa. *Journal of Fish Biology*, **59**, 593-604.

Tichy, H., Seegers, L. (1999). The *Oreochromis alcalicus* flock (Teleostei: Cichlidae) from Lakes Natron and Magadi, Tanzania and Kenya: a model for the evolution of "new" species flocks in historical times? *Ichthyological Exploration of Freshwaters*, **10**, 147-174.

Townsend, C.R. (2003). Individual, population, community, and ecosystem consequences of a fish invader in New Zealand streams. *Conservation Biology*, **17**, 38-47.

Trapani, J. (2003). Morphological variability in the Cuartro Cienegas cichlid, *Cichlasoma minckleyi*. *Journal Fish Biology*, **62**, 276-298.

Tregenza, T. & Butlin, R.K. (1999). Speciation without isolation. *Nature* **400**, 311-312.

Trendall, J. (1988). The distribution and dispersal of introduced fish at Thumbi West Island in Lake Malawi, Africa. *Journal of Fish Biology*, **33**, 357-369.

Trewavas, E. (1931). A revision of the cichlid fishes of the genus *Lethrinops*, Regan. *Annals and Magazine of Natural History, ser. 10*, **7**, 133-152.

Trewavas, E. (1935). A synopsis of the cichlid fishes of Lake Nyasa. *Annals and Magazine of Natural History, ser. 10*, **16**, 65-118.

Turner, G.F. (1994). Fishing and the conservation of the endemic fishes of Lake Malawi. *Archive für Hydrobiologie Beiheft, Ergebnisse der Limnologie*, **44**, 481-494.

Turner, G,F. (1996). *Offshore cichlids of Lake Malawi*. Cichlid Press, Lauenau, Germany.

Turner, G.F. (2000). The Nature of Species in Ancient Lake: Perspectives from the Fishes of Lake Malawi. *Advances in Ecological Research* **31**, 39-60.

Turner, G.F. (2002). The nature of species in ancient lakes: perspectives from the fishes of Lake Malawi. *Advances in Ecological Research*, **31**, 39-60.

Turner, G. F. & Burrows, M. T. (1995). A model of sympatric speciation by sexual selection. *Proceedings of the Royal Society, London Series B* **260**, 287-292.

Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J., and Robinson, R. L. (2001). How many species of cichlid fishes are there in African lakes? *Molecular Ecology* **10**, 793-806.

Vandewoestijne, S. & Baguette, M. (2004). Demographic versus genetic dispersal measures. *Population Ecology*, **46**, 281-285.

van Doorn, G. S., Noest, A. J. & Hogeweg, P. (1998). Sympatric speciation and extinction driven by environment dependent sexual selection. *Proceeding of the Royal Society, London, series B* **265**, 1915-1919.

Volpato, G.L., Luchiari, A. C., Duarte, C. R. A., Barreto, R. E., Ramanzini, G. C. (2003). Eye color as an indicator of social rank in the fish Nile tilapia. *Brazilian Journal of Medical and Biological Research* **36**, 1659-1663.

Vos, P., Hogers, R., Bleeker, M., *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407-4414.

Wainwright, P.C. (1996). Ecological Explanation through Functional Morphology: The Feeding Biology of Sunfishes. *Ecology*, **77**, 1336-1343.

Wagner, W. E., Jr. (1998). Measuring female mating preferences. *Animal Behaviour* **55**, 1029-1042.

Walker, J. (2000) MORPHOMETRIKA: *Geometric Morphometric Software for the Power Macintosh*, www.usm.maine.edu/~walker/software.html.

Wang, Z., Baker, A.J., Hill, G.E., Edwards, S.V. (2003). Reconciling actual and inferred population histories in the house finch (*Carpodacu mexicanus*) by AFLP analysis. *Evolution*, **57**, 2852-2864.

Werner, N. Y. & Lotem, A. (2003). Choosy males in a haplochromine cichlid: first experimental evidence for male mate choice in a lekking species. *Animal Behaviour* **66**, 293-298.

West-Eberhard, M.J. (1983). Sexual selection, social competition, and speciation. *Quarterly Review of Biology*, **58**, 155-183.

Westneat, M.W. (1995). Feeding, function, and phylogeny: Analysis of historical biomechanics and ecology in labrid fishes using comparative methods. *Systematic Biology*, **44**, 361-383.

Wickler, W. (1962). "Egg-dummies" as natural releasers in mouth-breeding cichlids. *Nature*, **194**, 1092-1093.

Witte, F. (1984). Consistency and functional significance of morphological differences between wild-caught and domestic *Haplochromis squamipinnis* (Pisces, Cichlidae). *Netherlands Journal of Zoology*, **34**, 596-612.

Witte, F., Goldschmidt, T., Wanink, J., van Oijen, M, Goudswaard, K, Witte-Maas, E., & Bouton, N. (1992) The destruction of an endemic species flock: Quantitative data on the decline of the Haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes*, **34**, 1-28.

Won, Y.-J., Sivasundar, A., Wang, Y., Hey, J. (2005). On the origin of Lake Malawi cichlid species: A population genetic analysis of divergence. *Proceedings of the National Academy of Sciences* **102**, 6581-6586.

Wong, B. B. M. (2004). Superior fighters make mediocre fathers in the Pacific blue-eyed fish. *Animal Behaviour* **67**, 583-590.

Wong, B. B. Keogh, M., J. S. & Jennions, M. D. (2004). Mate recognition in a freshwater fish: geographical distance, genetic differentiation, and variation in female preference for local over foreign males. *Journal of Evolutionary Biology* **17**, 701-708.

Wright, S.J., Carrasco, C., Calderon, O., Paton, S. (1999). The El Niño southern oscillation, variable fruit production, and famine in a tropical forest. *Ecology*, **80**, 1632-1647.

Wu, C-I. (1985). A stochastic simulation study on speciation by sexual selection. *Evolution*, **39**, 66-82.

Zimmerer, E.J. & Kallman, K.D. (1988). The inheritance of vertical barring (aggression and appeasement signals) in pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae, Teleostei). *Copeia*, **1988**, 299-307.

APPENDIX A

UNIVERSITY OF NEW HAMPSHIRE

Office of Sponsored Research
Service Building
51 College Road
Durham, New Hampshire 03824-3585
(603) 862-3564 FAX

LAST NAME	Carleton	FIRST NAME	Karen
DEPT	Zoology Department, 212 Rudman Hall	APP'L DATE	12/13/2000
OFF-CAMPUS ADDRESS (if applicable)	Zoology Department, 212 Rudman Hall	IACUC #	001201
		REVIEW LEVEL	2
		Today's Date	1/18/2001
PROJECT TITLE	Genetics of cichlid visual system		

All cage, pen or other animal identification records must include your IACUC Protocol # as listed above.

The Institutional Animal Care and Use Committee has reviewed and approved the protocol submitted for this study under Category 2 on Page 3 of the "Application for Review of Animal Use or Instruction Protocol" – the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

For the Institutional Animal Care and Use Committee,

Suzanne Mitchell

Suzanne H. Mitchell, Ph.D.
Chair, Institutional Animal Care and Use Committee

cc: File
        Thomas Kocher, Co-PI

178