

Sexual Selection and Genetic Relationships of a Sibling Species Complex of Bower Building Cichlids in Lake Malawi, Africa

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Abstract Protein electromorphs of five taxa of the sand dwelling genus *Tramitichromis* from Lake Malawi were examined by starch-gel electrophoresis. Twenty-four enzyme loci were examined. No diagnostic alleles were discovered which distinguished the different taxa; however, nine loci were polymorphic in at least one taxa with unbiased mean heterozygosities ranging from 6.9 to 12.6%. The bower form of these five taxa, which earlier have been referred to as sibling species was also analyzed. Results from this study provide evidence that bower form in these cichlid taxa is correlated with genetic distance measurements. The data suggest that the taxa are isolated 'sibling' species, which recently diverged, or that they are 'incipient' species with minimal gene flow between bower building morphs. The congruence of the allozyme data suggests a genetic basis for bower form. Variation in bower form is hypothesized to arise from nonadaptive differences in female choice, which leads to reproductively isolated species as predicted by Fisher's model of runaway sexual selection.

Sexual selection may be a major factor in the proliferation of polygamous species (Lande, 1981). When males provide no resources or parental care and females can mate with numerous males, 'extravagant' male secondary characteristics may result exclusively from sexual selection (Darwin, 1871). These extreme characters could result from a positive 'runaway' feedback loop due to the joint evolution of female mate choice and the secondary sexual attributes of the male. Fisher (1930) hypothesized that "an evolution of sexual preference due to this cause would establish an effective isolation between two differentiated parts of a species, even when geographical and other factors were least favorable to such separation."

Characters subject to high variance in female choice such as the behavior of a male during courtship could change quickly (Lande, 1981). Divergence in male courtship might develop rapidly and repeatedly due to local population differences in female choice (Lande, 1982). Lek-breeding species especially should exhibit "extraordinary geographic variation in male attributes" (Arnold, 1983). Lek-

ing cichlids (McKaye, 1984) of the species-rich African Great Lakes, therefore, appeared to be an ideal vertebrate group to study this Fisherian 'runaway' process and its role in speciation (Dominey, 1984; McKaye, 1991). Modes of cichlid speciation in the three Great Lakes of Africa have long been debated, yet no general mechanism has emerged to explain the amazing diversity of cichlids (Fryer and Iles, 1972; Greenwood, 1982; Keenleyside, 1991). Forms which were thought to be color morphs of a single species have been identified by morphological (McKaye and Stauffer, 1986), behavioral (Holzberg, 1978), and genetic data (McKaye et al., 1982, 1984) to be reproductively isolated. An adaptive model of sympatric speciation by disruptive selection has been developed to explain the proliferation of some rock-dwelling species (McKaye, 1980), but speciation due to sexual selection might be a more parsimonious explanation for the explosive speciation of these colorful fish (Dominey, 1984; McKaye, 1991).

Differences in breeding color among males are associated with reproductive isolation among taxa (Holzberg, 1978; McKaye et al., 1984). This conclu-

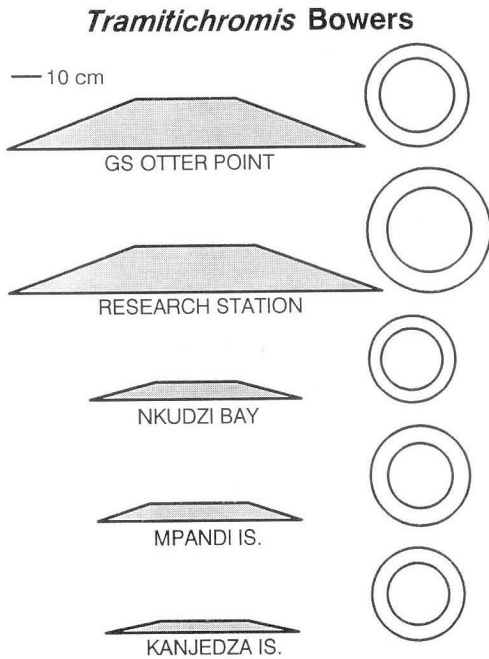


Fig. 1. Mean bower dimensions of five taxa of the genus *Tramitichromis*. The side view shows, base and top diameters, slope and height of bower. The circle shows the top diameter with the inner platform diameter. All measures with significantly different $P < 0.05$ (ANOVA D.F. 4, 99: base $F = 50.8$, top diameter $F = 25.9$; slope $F = 55.6$, height $F = 55.5$; platform diameter $F = 27.7$; top slope $F = 8.7$, see text for discussion).

sion for the Lake Malawi cichlids, however, is based primarily upon the rock-dwelling cichlids, the *mbuna*. Several hundred cichlid species inhabit the sand and weed environments, constituting 80% of Lake Malawi's western shoreline (McKaye and Gray, 1984). Nevertheless, males of sibling species can be easily distinguished by the form of the bower they construct (McKaye, 1984, 1991; Bass, 1988).

Bowers are sites for both courtship and spawning. They are solely display sites, and are not used in care of eggs or young. A female lays her eggs in the bower, but immediately picks them up in her mouth where the eggs are subsequently fertilized. The female leaves the arena after all of the eggs are laid. Males engage in no parental care, so the form and function of these breeding arenas are analogous to bird leks (McKaye, 1983). Considerable energy is expended in building these bowers, which can range in size from small depressions in the sand to craters

over 3 m in diameter (McKaye and Stauffer, 1988; McKaye, 1991) and no adaptive explanation for the diversity of bower forms is apparent. Females of some species such as *Copadichromis eucinostomus* (Regan) choose males which have the largest bowers (McKaye et al., 1990). For males of *Otopharynx argyrosoma* (Regan) small depressions suffice for a display site, and females choose males in the center of the arena (McKaye, 1991).

Bower form has been hypothesized to be a central component to female mate choice and the reproductive behavior of sand-dwelling cichlids leading to genetic isolation of sibling species (Dominey 1984; McKaye, 1984, 1991). If bower form is heritable, or if species specific habitat selection is strongly affected by historical legacy, then a phylogeny of sibling species based on bower forms should be congruent with one derived from genetic analysis. No such congruence would be expected, however, if the form of the bower is due to random local ecological conditions leading to adaptive responses in bower building or variance among populations which is non-heritable due to substrata texture and sand particle size.

We focused on five taxa of a group of sand-dwelling species previously referred to as the *Lethrinops lituris* group (Bass, 1988, 1990) and now placed in the genus *Tramitichromis* (Eccles and Trewavas, 1989). The bowers of all species in this group are cone-shaped with a flat breeding platform on top and a short rim around the platform (see Bass [1988] for photograph). Males construct bowers by picking up mouthfuls of sand and depositing the sand in what appears to be a species specific pattern (McKaye, 1983, 1984, 1991).

Material and Methods

Bowers

Bower dimensions (height, slope, base diameter, top diameter, and platform) were measured for a minimum of 20 bowers of each taxa (Fig. 1) at five different localities around the Nankumba Peninsula, Golden Sands Otter Point (15 m depth), Fisheries Research Station (7 m depth), Kanjedza Island (3–4 m depth), Mpandi Island (4–5 m depth), and Nkudzi Bay (5 m depth) (see Fig. 1 in McKaye [1981] for map of area).

Differences in bower shape were analyzed using

Bower Building Cichlids

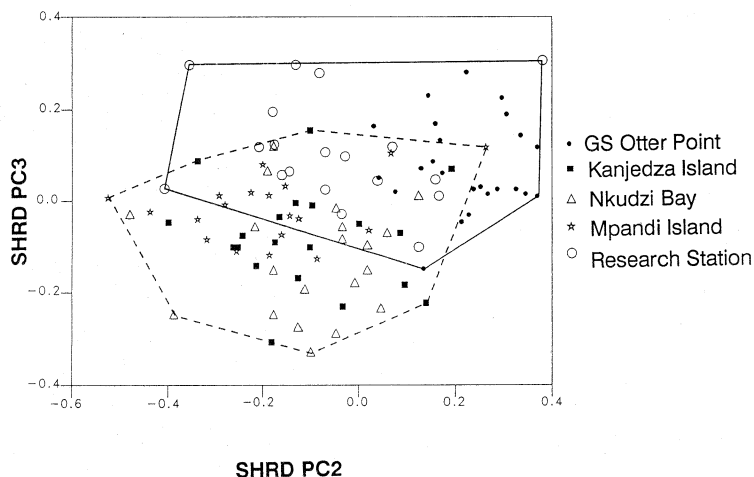


Fig. 2. Plot of the sheared principal components of the bower forms of *Tramitichromis* species. The MANOVA showed that clusters formed by plotting the second sheared principal component against the third sheared principal component for each taxa were significantly different ($P < 0.05$). GS Otter Point and Research Station form one group, while Nkudzi Bay, Mpandi Island, and Kanjedza Island form another (see text for discussion).

sheared principal components analysis (PCA) (Humphries et al., 1981; Bookstein et al., 1985). This analysis restricts the variation due to size of the first component, thus subsequent components are strictly shape related. Bowers of the taxa were compared by plotting the sheared second principal component against the sheared third principal component. The clusters formed by each taxa were analyzed using multivariate analysis of variance (MANOVA). Additionally, differences among dimensions were tested using an ANOVA in conjunction with Duncan's multiple range test.

Genetics

Protein electromorphs for the five taxa were examined using standard horizontal starch-gel electrophoresis and histochemical staining procedures (Allendorf et al., 1977; Aebersold et al., 1987). The fish used were captured by being herded into a fine mesh net at all study sites. Only males occupying a bower or females circling with males in a bower were captured.

The liver, muscle, heart, and eyes of adult fish were removed and stored in liquid nitrogen for 2 weeks before being transported to the laboratory for analysis. Samples were run without knowledge of the presumed relationships based on bower form. All calculations based on gene frequencies were per-

formed using BIOSYS-I (Swofford and Selander, 1981).

Results

Bowers

Bower measurements for all dimensions were significantly different (Fig. 1, ANOVA $P < 0.05$). The MANOVA showed that clusters formed by plotting the second sheared principal component against the third sheared principal component for each taxa were significantly different ($P < 0.05$). On the basis of bower structure, GS Otter Point and Research Station make up one group, while Nkudzi Bay, Mpandi Island, and Kanjedza Island form another (Fig. 2).

Independently, the clusters were significantly different ($P < 0.05$) along both the sheared second principal component and the sheared third principal component: thus a Duncan's multiple range test could be used to determine significant differences among taxa. Along the second sheared principal component, GS Otter Point was significantly ($P < 0.05$) different from all of the other taxa; Research Station, Nkudzi and Kanjedza Island were not significantly different from each other. Moreover, along the third sheared principal component, Research Sta-

tion and GS Otter Point were not significantly different from each other but were significantly ($P < 0.05$) different from all other taxa; Mpandi Island and Kanjedza Island were not significantly different from each other; and, Nkudzi Bay was significantly different from all other taxa.

When each of the dimensions were analyzed separately, GS Otter Point and Research Station were not significantly different in base, slope and height from each other, but were different from all the other taxa; and, Mpandi Island, Kanjedza Island and

Nkudzi Bay were not significantly different in these three dimensions. Nkudzi Bay was significantly different ($P < 0.05$) from Mpandi Island and Kanjedza Island in platform diameter. Research Station was significantly ($P < 0.05$) from GS Otter Point on platform diameter.

From this analysis two major groupings are evident: GS Otter Point and Research Station are distinct from Nkudzi Bay, Mpandi Island and Kanjedza Island. Nkudzi Bay on the basis of platform differences can be distinguished from Mpandi Island and

Table 1. Gene frequencies at polymorphic loci and measures of genetic variation in five taxa of *Tramitichromis*

Locus	Research S.	GS Otter	Nkudzi B	Mpandi Is.	Kanjedza Is.
ADA					
a	0.000	0.125	0.250	0.050	0.050
b	0.056	0.438	0.125	0.400	0.300
c	0.333	0.281	0.292	0.275	0.350
d	0.250	0.094	0.083	0.175	0.150
e	0.333	0.031	0.083	0.050	0.150
f	0.028	0.031	0.167	0.050	0.000
FH					
a	1.000	1.000	0.889	1.000	1.000
b	—	—	0.056	—	—
c	—	—	0.056	—	—
GPI-1					
a	0.875	0.906	0.722	0.452	0.727
b	0.125	0.094	0.278	0.548	0.273
GPI-3					
a	1.000	1.000	0.944	1.000	0.956
b	—	—	0.056	—	0.044
G3PDH					
a	0.947	0.867	0.861	0.750	0.500
b	0.053	0.133	0.139	0.250	0.500
sIDHP					
a	1.000	1.000	0.944	1.000	1.000
b	—	—	0.056	—	—
LDH-3					
a	0.625	0.906	0.417	0.595	0.455
b	0.025	0.000	0.333	0.238	0.273
c	0.000	0.063	0.250	0.119	0.273
d	0.350	0.031	0.000	0.048	0.000
sMEP					
a	0.950	0.719	0.833	0.929	0.727
b	0.050	0.281	0.167	0.071	0.273
PGM					
a	1.000	1.000	1.000	1.000	0.955
b	—	—	—	—	0.045
N	20	16	18	21	11
Mean Het. (expected)	0.069	0.072	0.120	0.098	0.126
% Polymorphism	20.8	20.8	33.3	20.8	29.2

Kanjedza Island.

Genetics

The electrophoretic data are interpreted throughout according to minimal genetic Mendelian assumptions. Each enzyme system is treated as the product of a single genetic locus, with differing electrophoretic mobilities indicating the existence of allomorphs.

Fifteen of the 24 loci (nomenclature from Shaklee et al., 1989; locus designations follow enzyme names and IUACN numbers) studied did not vary in our survey: Aspartate aminotransferase (2.6.1.1)-AAT-1; Adenylate kinase (2.7.4.3)-AK; Fructose-biphosphate aldolase (4.1.2.13)-FBALD; Glucose-6-phosphate isomerase (5.3.1.9)-GPI-2; Lactate dehydrogenase (1.1.1.27)-LDH-1, LDH-2; Malate dehydrogenase (1.1.1.37)-mMDH, sMDH-1, sMDH-2; Mannose-6-phosphate isomerase (5.3.1.8)-MPI; Dipeptidase (3.4.-.-)-PEPA; Tripeptide aminopeptidase (3.4.-.-)-PEPB and Triose phosphate isomerase (5.3.1.1)-TPI-1, TPI-2, TPI-3. Nine loci were found to be polymorphic: Adenosine deaminase (3.5.4.4)-ADA; Fumate hydratase (4.2.1.2)-FH; Glycerinaldehyde-3-phosphate dehydrogenase (1.2.1.12)-G3PDH; Glucose-6-phosphate isomerase (5.3.1.9)-GPI-1, GPI-3; Isocitrate dehydrogenase (1.1.1.42)-sIDHP; Lactate dehydrogenase (1.1.1.27) LDH-3; Malic enzyme (1.1.1.40)-sMEP and Phosphoglucomutase (5.4.2.2)-PGM.

Within population samples, the polymorphic loci were all found to be in Hardy Weinberg equilibrium, and there were no significant differences ($P > 0.05$) in gene frequencies between the sexes. None of the loci examined were fixed for alternative alleles. However, allele frequencies were heterogeneous among taxa for five of the polymorphic loci—ADA, G3PDH, GPI-1, LDH-3, sMEP ($P < 0.05$; G test, see Table 1).

The mean F_{st} for all taxa was 0.101 and the modified Roger's genetic distances (Wright, 1978) ranged from 0.093 to 0.140 (Table 2). Examination of the modified Roger's genetic distances indicated two distinct groupings. Fishes from the Research Station and GS Otter Point were similar to each other (0.111 units), but distinct (0.121–0.140 units) from Mpandi Island and Kanjedza Island (Table 2). Fishes from Nkudzi Bay, Mpandi Island, and Kanjedza Island were similar to each other (0.093–0.095 units) forming a second grouping. However, Nkudzi

Bay fishes (Table 2) displayed an intermediate relationship to both Research Station and GS Otter Point (0.114–0.116), while Mpandi and Kanjedza Island fishes were distinct from the Research Station and GS Otter Point.

Discussion

The results of this study, as did earlier studies on rockdwelling cichlids (McKaye et al., 1982, 1984) indicate that there are no fixed alternative alleles at any of the loci we examined. Therefore, we found no clear diagnostic electromorph character to separate and identify these taxa. The results obtained are consistent with either the taxa being sibling species or polymorphic for bower form. Because electrophoretic divergences are time dependent (Corruccini et al., 1980), sibling species that have recently diverged may show little differentiation in electromorphs (McKaye et al., 1984).

Results from this study provide evidence that bower form in these cichlid taxa is correlated with genetic distance measurements. The congruence of the allozyme data suggests either a direct genetic basis for bower form or taxa specific habitat selection affected by historical legacy. We hypothesize that variation in bower form among taxa arises from nonadaptive differences (Arnold, 1983; McKaye, 1991). Such variation could lead to the formation of reproductively isolated species as predicted by Fisher's model of runaway sexual selection (Lande, 1981).

Earlier studies (McKaye, 1983; McKaye et al., 1990) of *Copudichromis eucinostomus* demonstrated that two conditions necessary for sexual selection are operating among cichlids: 1) variation in a characteristic, bower size, derived from differences in male behavioral traits, and 2) female use of this characteristic as a criterion in mating. Similar behavior results have been found with the *Tramitichro-*

Table 2. Matrix of modified Rogers genetic distance (Wright, 1978)

Taxa	1	2	3	4	5
1. Research Station	—				
2. GS Otter Point	0.111	—			
3. Nkudzi Bay	0.114	0.116	—		
4. Mpandi Island	0.129	0.121	0.093	—	
5. Kanjedza Island	0.140	0.122	0.095	0.095	—

mis taxa studied here (McKaye and Stauffer, unpubl.). The new genetic evidence of a correlation of genetic distance with bower form is consistent with models linking sexual selection with the explosive speciation of cichlid fishes of the Great Lakes of Africa (Dominey, 1984). The data, however, are only inferential in linking a genetic basis for bower building. The next test of the sexual-selection hypothesis of mate choice and cichlid speciation requires heritability studies of bower building among these closely related cichlid taxa.

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アフリカの馬拉ウ湖における営巣シクリッド同胞種群の遺伝的類縁関係と性選択

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馬拉ウ湖の砂底域に生息する *Tramitichromis* 属シクリッドの5グループのタンパク質多型を、デンブengel電気泳動法によって調べた。24酵素遺伝子座を調査したところ、9遺伝子座が少なくとも一つのグループで多型的であり、平均ヘテロ接合体率は6.9-12.6%であったが、これらのグループ間における遺伝子の置換は認められなかった。同胞種であると考えられているこれら5グループの巣の形態も併せて調査した結果、その類似性は遺伝的類似性とよく一致し、巣の形には遺伝的基礎があるものと考えられた。本研究の結果はまた、これらのグループが最近分化し相互に独立した「同胞種」か、あるいは相互にわずかな遺伝子流動しかなくなった「発端種」であることを示唆するものである。マウント型の変異は、雌の配偶者選択の非適応的差異によってもたらされたもので、それは、Fisherの性選択におけるランナウェイ・モデルが予測するように、生殖的に隔離した種を生じさせるものであると推察される。